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FOREWORD

The year 1977 was a milestone in the history of the Sabine Island, site of EPA's Environmental Research Laboratory, Gulf Breeze, Florida (ERL,GB). Our new aquatic toxicological laboratory dedicated on October 7 became a symbol of the modernization of the island where research was originally conducted in a former quarantine station built at the turn of the century.

In the dedicatory address, EPA Deputy Administrator Barbara Blum predicted that ERL,GB will make a "significant contribution to many critical issues currently facing the marine environment." "Our goals," Ms. Blum said, "must be not only to help correct the dangerous environmental mistakes that have been made in the past, but, more importantly, to honestly assess and effectively address the difficult environmental realities that confront our generation."

U.S. Representative Robert L.F. Sikes echoed Ms. Blum's optimism that our laboratory will contribute to the national effort to "create a better American for tomorrow." And Dr. Stephen J. Gage, Assistant EPA Administrator for Research and Development, forecast a significant role for ERL,GB in EPA's transition from the problems of the 60's to the problems of the 80's.

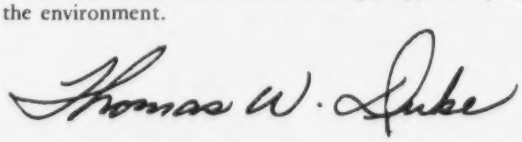
A symposium held in conjunction with our laboratory dedication ceremony drew representatives from national conservation groups, scientists, and professors who discussed the essential role of research and regulatory agencies in protecting marine ecosystems. Participants offered their views of the special function of federal agency scientists, the social responsibility of the scientists, and the need for research in support of environmental regulation.

A highlight of our Laboratory Dedication Ceremony was the presence of a scientific delegation from the Soviet Union, who took part in an American-Soviet bilateral exchange of environmental scientific technology. ERL,GB in 1977 participated in two projects sponsored under the American-Soviet Environmental Protection Agreement and also provided expertise for U.S. technical assistance efforts in Egypt and Poland.

With the enactment of Congressional amendments to the Federal Water Pollution Control Act and the Safe Drinking Water Act, ERL,GB research broadened in scope in 1977. Scientists who screened chemicals to determine their toxicity to marine species were joined by colleagues who examined how a test chemical traveled through the environment and how it may affect physical, chemical, and biological processes. One goal was to acquire a more thorough understanding of the biochemical mechanisms that are set in motion in organisms and in ecosystems under environmental stress.

In addition, new programs were initiated to examine the ecological impact of offshore oil drilling and the environmental acceptability of wastes from various manufacturing processes.

Investigations in 1977 focused on many subtle, but far-reaching effects of pollutants that threaten the ability of ecosystems to support vital life processes. A deeper knowledge of the intricacies of complex environmental interactions will afford new insights into threats posed by toxicants to man and life support systems. This report reviews ERL,GB's supportive research in 1977 for EPA's commitment to enhance the quality of life and to protect human health from the increasingly apparent problem of hazardous substances in the environment.



Thomas W. Duke
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16. ABSTRACT

This report summarizes results of aquatic research conducted by the Environmental Research Laboratory, Gulf Breeze, Florida, Office of Research and Development, U.S. Environmental Protection Agency from January 1 to December 30, 1977. The research program examines the impact of pesticides and other organic compounds on marine species and communities, and seeks to develop new methodology for determining ecological hazards of chemical substances under simulated natural conditions. Projects are outlined under four categories: research related to toxicological testing; biological processes and effects; development of offshore oil resources; and Kepone in the marine environment. Investigations conducted at the laboratory's Atlantic Coast field station at Bears Bluff, South Carolina, are also reviewed for the year 1977.

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NOTE: This report is for informational purposes only. All data and conclusions must be considered provisional. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Figure 1. ERL,GB scientists determine effects of pollutants on marine animals in a new toxicological laboratory dedicated in 1977

EXPERIMENTAL ENVIRONMENTS BRANCH

JACK I. LOWE, Chief

A new $1 million toxicological laboratory completed in 1977 provides EPA's Environmental Research Laboratory in Gulf Breeze, Florida, (ERL,GB) with expanded and modernized facilities for observing marine species exposed to toxic chemicals in raw, or filtered, flowing seawater (Fig. 1).

Tests conducted by the Experimental Environments Branch yield data required by EPA for registration and reregistration of pesticides, development of water quality standards, and the issuance of permits to dump wastes in the ocean.

Historically, ERL,GB research has been oriented toward an assessment of the effects of pesticides and other poisonous substances on the marine environment. In 1977, the Experimental Environments Branch established toxicity concentrations for individual pollutants on the basis of tests with single species of animals. Investigations extended from single species to multiple species of animals established as a community, and from criteria for lethal effects to more subtle, longer lasting effects of chemical contaminants and their fate and transport in the marine environment.

Fewer tests were conducted by the branch in 1977 on "hard" or organochlorine pesticides, and more tests were conducted on organophosphates and "third" generation pesticides that inhibit normal growth. The branch staff also initiated studies of complex industrial and municipal wastes.

The new aquatic laboratory will enable branch scientists to observe how marine life will respond to pollutants under natural or controlled conditions. Test animals (individually and in communities) are exposed to toxic compounds in raw or filtered flowing seawater. An intricate pumping system can deliver up to 450 gallons of seawater per minute from adjacent Santa Rosa Sound.

A wing of the laboratory houses ERL,GB's analytical chemistry section where pollutants and their concentrations are identified. The unit is equipped with 10 gas and 3 liquid chromatographs (some controlled by computers) for analyses of toxic compounds in marine water, sediment, and biota.

During 1977, the analytical chemistry section analyzed 3288 samples for pesticide and related organics. Analytical procedures were validated for trifluralin and two dealkylated products; Sevin[R] and 1-naphthol; mono- and dehydro-Kepone[R]; Lepthophos; pentachloronitrobenzene; Dimilin[R]; and EPN. In 1978, methods will be validated for determination of dimethoate, trichlorofon, Guthion[R], phorate, DEF[R], trithion, methylparathion, Altosid[R], and trichlorophenol in marine water and biota.

[R]Registered trademark

Acute Toxicity Tests (Dynamic)

STEVEN C. SCHIMMEL, Research Aquatic Biologist;
JAMES M. PATRICK, Jr., Biological Lab Technician

Data from acute toxicity tests have played a crucial role in EPA's development of Water Quality Criteria, evaluations of new, "substitute" chemicals and in Effluent Limitation Hearings.

In the past, research results have appeared on the caution label of pesticides tested with methods developed at ERL,GB. In 1977, acute toxic effects of sodium pentachlorophenate (PCP), Kepone, Leptophos, EPN, trifluralin and Sevin were investigated in flowing seawater tests with estuarine molluscs, crustaceans, and fish. Studies of the herbicide trifluralin and the insecticide Sevin will be completed in 1978; a manuscript reporting results of Leptophos/EPN research is in preparation.

Pentachlorophenol (PCP) and sodium pentachlorophenate (NA-PCP), found in defoliants, herbicides, insecticides, wood preservatives, and other products, were tested with several estuarine animals collected near ERL,GB. The 96-hour (96-h) LC50s (concentration estimated to be lethal to 50% of test organisms) for each organism tested were: grass shrimp (Palaemonetes pugio), 515 micrograms per liter (µg/ℓ); brown shrimp (Penaeus aztecus), > 195 µg/ℓ; longnose killifish (Fundulus similis), > 306 µg/ℓ; pinfish (Lagodon rhomboides), 53.2 µg/ℓ; and striped mullet, (Mugil cephalus), 112 µg/ℓ.

Oyster shell deposition data were analyzed by linear regression with probit transformation to determine an EC50 (concentration of PCP effective in reducing shell deposition of exposed oysters to 50% of that of control oysters) and 95% confidence intervals.

Sodium pentachlorophenate was acutely toxic to oysters (EC50 = 76.5 µg/ℓ) exposed for 96-h (Table 1). Due to the low seawater temperature, control oysters deposited an average of only 1 mm shell/oyster. Therefore, oysters were exposed for an additional 96-h, but the EC50 value remained unchanged.

Exposure of brown shrimp to Na-PCP concentrations as high as 195 µg/ℓ and the grass shrimp to concentrations up to 515 µg/ℓ did not lead to significant mortality.

Sodium pentachlorophenate, at the concentrations tested, was acutely toxic to striped mullet and pinfish but not to longnose killifish (Table 1). Pinfish were the

Table 1. Acute toxicity of sodium pentachlorophenate (Na-PCP) to several estuarine organisms

Species	Size[a] ($\bar{x}$, mm)	96-h µg/ℓ[b]		Temp. ($\bar{x}$, °C)	Salinity ($\bar{x}$, °/oo)
		Nominal	Measured		
Crassostrea virginica	45	104.0 (54-158)[c]	76.5 (37-116)	8.4	20.3
Penaeus aztecus	66	>320.0	>195.0	25.0	26.5
Palaemonetes pugio	18	>560.0	>515.0	24.8	24.3
Fundulus similis	42	>560.0	>306.0	24.4	22.9
Lagodon rhomboides	80	107.6 (93.7-122.0)	53.2 (42.4-65.4)	25.0	20.8
Mugil cephalus	58	221.6 (92.3-489.6)	112.1 (44.0-210.4)	24.7	25.5

[a]Size is height (umbo-distal valve edge) for oysters; rostrum-telson length for shrimp; standard length for fishes.
[b]Effect measured is shell deposition for oysters and mortality for shrimp and fishes.
[c]The 95% confidence intervals are in parentheses.

most sensitive species tested: the 96-h LC50 was 53.2 µg/ℓ.

Bioconcentration Tests

STEVEN C. SCHIMMEL, Research Aquatic Biologist;
JAMES M. PATRICK, Jr., Biological Laboratory Technician

The purpose of these tests is to determine the rate of uptake (mainly from water) of toxic organics and the rate at which these substances are depurated from estuarine mollusc and fish tissues. Resulting data can be used to indicate the bioconcentration factor (BCF) of toxic chemicals. BCF, the chemical concentration found in tissues of organisms divided by the exposure concentration in seawater, is useful in predicting the potential of a compound to persist and accumulate in marine food webs.

In 1977, two long-term studies were conducted to determine toxicity, uptake, and depuration of Kepone in blue crabs (Callinectes sapidus) after the James River in Virginia was contaminated by Kepone and closed to commercial fishing.

In the first study, Kepone was administered to crabs in seawater (0.03 or 0.3 µg/ℓ) or food (eastern oyster, Crassostrea virginica, containing 0.25 µg/g Kepone). Uptake of Kepone in 28 days was primarily through the contaminated oysters. When the crabs were held in Kepone-free seawater and fed Kepone-free oysters for 28 days, no loss of the insecticide was evident. Adverse effects on molting and survival were observed in crabs fed oysters that contained 0.25 µg/g Kepone.

The second study investigated: (1) the depuration of Kepone over a 90-day period in blue crabs fed oysters (containing 0.15 µg/g Kepone) from the James River, Virginia; and (2) the effects of Kepone on molting and survival of blue crabs fed James River oysters or laboratory-contaminated oysters that contained 0.15 or 1.9 µg/g Kepone. Crabs fed Kepone-contaminated oysters followed by a diet of Kepone-free oysters for 90 days had detectable concentrations of the insecticide in tissues. Blue crabs that ate oysters containing Kepone in concentrations similar to those found in oysters from the James River died in greater numbers or molted less frequently than crabs fed Kepone-free oyster meats.

In bioconcentration studies with PCP, eastern oysters exposed to measured PCP concentrations of 25.0 and 2.5 µg/ℓ for 28 days accumulated the chemical in their tissues an average of 41 and 78 times, respectively (Fig. 2). Pentachlorophenol reached an apparent equilibrium in oysters within the first four days in both exposure concentrations and remained relatively constant throughout the uptake portion of the study. The mean concentration of PCP in oysters from the 25 µg/ℓ aquarium was 1060 µg/kg and 180 µg/kg for the 2.5 µg/ℓ aquarium. After the PCP delivery was discontinued, however, the oysters purged themselves of the pesticide within four days.

In summary, Na-PCP reduced shell deposition of eastern oysters at concentrations >34 µg/ℓ during a 192-h exposure. Concentrations of the pesticide as high as 195 and 515 µg/ℓ, however, were neither toxic to brown shrimp nor grass shrimp, respectively, in 96-h exposures, nor did the animals bioconcentrate the chemical. Pinfish were the most sensitive of all species tested (96-h LC50 = 53 µg/ℓ). Oysters bioconcentrated PCP 41 to 78 times the amount measured in water and, when held in PCP-free seawater, depurated the chemical to nondetectable concentrations in four days. Compared with other organochlorine insecticides (such as toxaphene, chlordane, and BHC) tested on estuarine animals, Na-PCP is relatively less toxic and is bioconcentrated to a lesser extent.

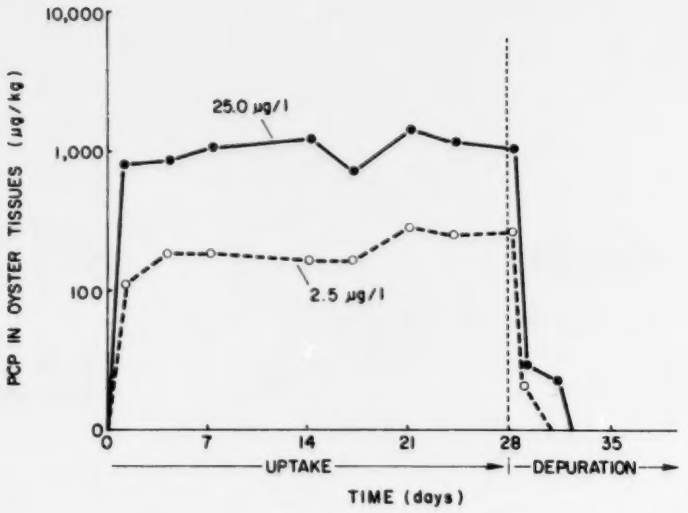

Figure 2. Uptake of pentachlorophenol (PCP) by eastern oysters (Crassostrea virginica) exposed 28 days, then allowed to depurate in PCP-free seawater *

Chronic Toxicity Tests

DAVID J. HANSEN, Research Aquatic Biologist;
LARRY R. GOODMAN, Research Biologist;
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Long-term toxicity tests with saltwater species primarily have used juveniles that are found inshore and are easily collected. Use of saltwater fish and invertebrates in life-cycle tests has been limited because of difficulties related to their culture in the laboratory.

In 1977, ERL,GB researchers successfully maintained sheepshead minnows (Cyprinodon variegatus) in life-cycle tests to determine maximum acceptable toxicant concentrations (MATC) of pollutants and application factors that can be used to provisionally estimate a chronically safe toxicant concentration applicable to species when only acutely lethal concentrations are known. (The application factor [AF] is the ratio between the MATC in a life-cycle test and the acute or incipient LC50).

Sheepshead minnows were used in 1977 in embryo/juvenile and partial and entire life-cycle tests (Fig. 3) to determine effects of long-term exposures during sensitive life stages to Leptophos, trifluralin, trifluralin decomposition products, and Kepone.

Leptophos - Sheepshead minnows were exposed to Leptophos for 28 days in beginning with embryos and lasting through hatching and growth of fry to the juvenile stage. Survival of embryo, fry, and juvenile sheepshead minnows exposed to Leptophos for 28 days did not differ significantly from that of controls (Table 2). However, fish in 9.8 µg/ℓ were markedly less active from day 7 to 19, and were significantly shorter than control fish; survival seemed to be reduced. The highest concentration at which no effects were apparent (2.9 µg/ℓ) was greater than 0.1 of a concentration acutely lethal to 90% of the juvenile fish (20 µg/ℓ). This finding suggests that the chronic toxicity of Leptophos may not be excessive in relation to other insecticides.

Trifluralin - Sheepshead minnows were exposed to trifluralin (2,6-dinitro-N, N-dipropyl-α,α,α-trifluro-p-toluidine) for 28 days from the embryonic stage through hatching and growth of fry to the juvenile stage. Acute toxicity tests also were conducted on trifluralin, trifluralin III, and other decomposition products.

Trifluralin was found to be more toxic to sheepshead minnows in acute, or in embryo-to-juvenile tests, than the decomposition products tested. The 96-h LC50 of Trifluralin to juvenile fish in static tests was 143 µg/ℓ (95% confidence limit = 117-175 µg/ℓ). Survival and growth of

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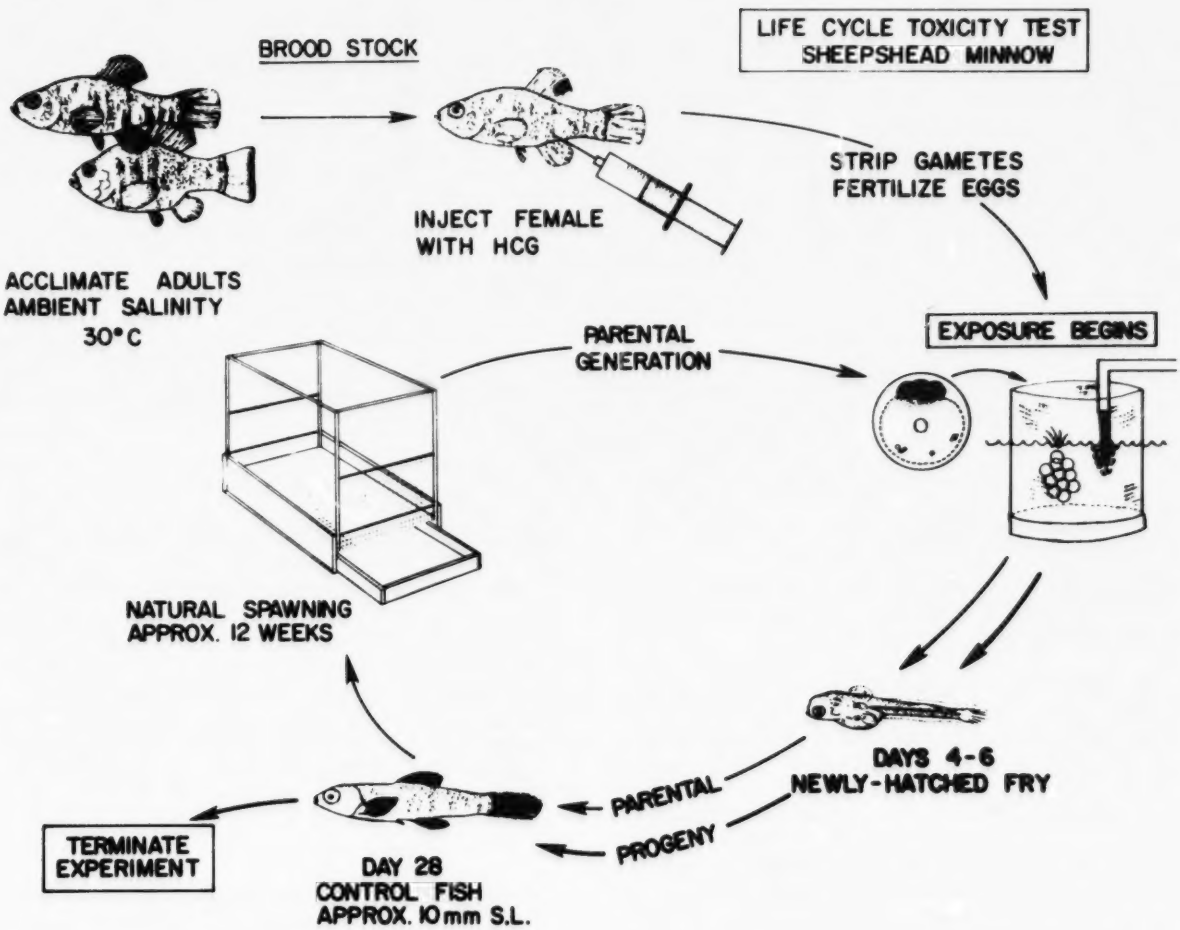

Figure 3. Flow chart of a life-cycle toxicity test with the sheepshead minnow

Table 2. Toxicity of Leptophos to sheepshead minnows (Cyprinodon variegatus) exposed continuously in a 28-day test. The test began with 80 embryos in each concentration and lasted through hatching and growth of fry to juvenile fish

Concentration		Percentage	Combined Embryo/	Average Standard
Nominal	Measured	Hatching	Fry Survival, %	Length, mm
Control/ Carrier	ND	69	68	9.4
0.93	0.33	67	66	9.1
1.9	2.3	69	68	9.0
3.8	2.4	60	59	9.7
7.5	2.9	57	56	9.6
15.	9.8	70	62	8.0

ND = non-detectable < 0.02 µg/ℓ

fish in 20 or 31 µg/ℓ of trifluralin in the embryo-to-juvenile test was significantly reduced, but exposure to 1.2, 2.7, or 5.5 µg/ℓ affected neither growth nor survival.

Signs of poisoning (lethargy, upset equilibrium, edema, and melanism of the posterior one-third of the body) increased in severity and frequency of occurrence as concentrations increased from 2.7 to 31 µg/ℓ. In the initial test and a second experiment, light microscopy revealed pathological effects in fish that showed external signs of poisoning.

Concentrations of trifluralin plus trifluralin II, (2,6-dinitro-n-propyl-α,α,α-trifluoro-p-toluidine) in juvenile sheepshead minnows on day 28 ranged from 500 to 2,200 times the concentration of trifluralin measured in the exposure water.

Trifluralin III was toxic to juvenile sheepshead minnows in acute static tests at nominal concentrations of about 1 mg/ℓ but exposure to measured concentrations of from 1.8 to 29 µg/ℓ in the 28-day tests produced no visible signs of poisoning, decrease in growth, or decrease in survival that were not observed in control fish. Concentrations of trifluralin III accumulated by fish were similar to those measured in the exposure water.

Kepone - The effect of Kepone on survival, growth, and reproduction was studied in sheepshead minnows throughout an entire life cycle in these concentrations: 0.0 (control), 0.01, 0.02, 0.06, 0.12, 0.37, or 0.77 µg/ℓ (Fig. 3). Signs of Kepone poisoning were scoliosis, lordosis, blackened tails, edema, decreased growth, a reduction in eggs spawned, and death. Kepone was found in the fish tissues in all lifestages. (Pathological effects of Kepone in this test and a second exposure are described on p. 55.

Chronic Toxicity of Methoxchlor, Malathion, and Carbofuran to Sheepshead Minnows (Cyprinodon variegatus)

P. R. PARRIS, Principal Investigator. EPA Contract 68-03-0264. Bionomics, EG&G, Inc.
DAVID HANSEN, Project Officer

Sheepshead minnows (Cyprinodon variegatus) were exposed to each of three pesticides--methoxychlor, malathion, and carbofuran--in flowing seawater to determine the acute and chronic (partial life-cycle) effects. The calculated 96-h LC50's and 95% confidence limits, based on measured concentrations, were: methoxychlor, 49 micrograms per liter (µg/ℓ), 37-65 µg/ℓ; malathion, 51 µg/ℓ, 41-63 µg/ℓ; and carbofuran, 386 µg/ℓ, 311-480 µg/ℓ.

Mortality of adult sheepshead minnows exposed to mean measured concentrations of methoxychlor $\geq$ 23 µg/ℓ was significantly (P 0.05) greater than mortality of control fish during the 140-day study. Further, hatching success of fry from eggs spawned by fish exposed to 23 µg/ℓ was significantly less than hatching success of control fry. The maximum acceptable concentration (MATC) was estimated to be >12<23 µg/ℓ and the application factor limits were 0.24-0.47.

Mortality of adult sheepshead minnows exposed to mean measured concentrations of malathion $\geq$ 18 µg/ℓ was significantly greater than mortality of control fish during the 140-day study. Mortality of fry hatched from eggs spawned by fish exposed to 9 and 18 µg/ℓ was significantly greater than mortality of control fry. The MATC was estimated to be >4<9 µg/ℓ and the application factor limits were 0.08-0.18.

Mortality of adult sheepshead minnows exposed to mean measured concentrations of carbofuran $\geq$ 49 µg/ℓ was significantly greater than mortality of control fish during the 131-day study. Hatching success of fry from eggs spawned by fish exposed to 49 µg/ℓ was significantly less than hatching success of control fry. Also, mortality of fry hatched from eggs spawned by fish exposed to 49 µg/ℓ was significantly less than hatching success of control fry. Also, mortality of fry hatched from eggs spawned by fish exposed to 23 and 49 µg/ℓ was significantly greater than control fry mortality. The MATC was estimated to >15<23 µg/ℓ and the application factor limits were 0.04-0.06.

Results of the study were published in the EPA Ecological Research Series, EPA-600/3-77-059, May 1977. (See Table 3, below).

Chronic Toxicity of Chlordane, Trifluralin, and Pentachlorophenol to Sheepshead Minnows (Cyprinodon variegatus)

P. R. PARRISH, Principal Investigator. EPA Contract 68-03-0264. Bionomics, EG&G, Inc.
DAVID HANSEN, Project Officer

Sheepshead minnows (Cyprinodon variegatus) were exposed to each of three chemicals--chlordane, trifluralin, or pentachlorophenol--in flowing, natural seawater to determine acute and chronic (full life-cycle) effects. The calculated 96-h LC50's and 95% confidence limits, based on measured concentrations, were: chlordane, 12.5 micrograms per liter (µg/ℓ), 3.4-45.9 µg/ℓ; trifluralin, 190 µg/ℓ, 128-282 µg/ℓ; and pentachlorophenol, 442 µg/ℓ, 308-635 µg/ℓ.

In a chronic test, sheepshead minnows were exposed to mean measured concentrations of chlordane (0.5-18.0 µg/ℓ) for 189 days. Exposure to concentrations $\geq$ 2.8 µg/ℓ caused significant ($P < 0.5$) mortality of parental fish. Exposure to chlordane concentrations $\geq$ 0.8 µg/ℓ significantly reduced hatch of embryos spawned by parental fish and exposure to concentrations $\geq$ 1.7 µg/ℓ caused significant mortality of second generation fish. The estimated maximum acceptable toxicant concentration (MATC) of chlordane for sheepshead minnows was >0.5<0.8 µg/ℓ; the application factor (AF) limits were 0.04-0.06.

Sheepshead minnows were exposed to mean measured concentrations of trifluralin (1.3-34.1 µg/ℓ) for 166 days. Exposure to concentrations $\geq$ 17.7 µg/ℓ caused significant mortality of parental fish. Exposure to trifluralin concentrations $\geq$ 9.6 µg/ℓ significantly reduced growth of parental fish and exposure to concentrations $\geq$ 4.8 µg/ℓ significantly reduced fecundity of parental fish. Exposure to concentrations $\geq$ 9.6 µg/ℓ significantly reduced hatch of embryos spawned by parental fish, and survival of and growth of second generation fish. The estimated MATC of trifluralin for sheepshead minnows was >1.3<4.8 µg/ℓ; the AF limits were 0.007-0.025.

Sheepshead minnows were exposed to mean measured concentrations of pentachlorophenol (18-389 µg/ℓ) for 151 days. Exposure to concentrations $\geq$ 88 µg/ℓ caused significant mortality of parental fish. Exposure to pentachlorophenol concentrations $\geq$ 195 µg/ℓ significantly reduced hatch of embryos spawned by parental fish and survival of second generation fish. The estimated MATC of pentachlorophenol for sheepshead minnows was >47<88 µg/ℓ; the AF limits were 0.11-0.20.

Results of these experiments will be reported in the EPA Ecological Research Series, EPA-600/3-78-010, in January 1978. (See Table 3.)

Table 3. Concentrations (µg/ℓ) of Six Pesticides Toxic to Sheepshead Minnows in Acute and Chronic Tests, and the Relationship of Acute Toxicity to Chronic Toxicity

Pesticide	96-h LC50 (95% confidence limits)	MATC limits	Application factor limits[a]
Methoxychlor	49 (37-65)	>12<23	0.24-0.47
Malathion	51 (41-63)	>4<9	0.08-0.18
Carbofuran	386 (311-480)	>15<23	0.04-0.06
Chlordane	12.5 (3.4-45.9)	>0.5<0.8	0.04-0.06
Trifluralin	190 (128-282)	>1.3<4.8	0.007-0.025
Pentachlorophenol	442 (308-635)	>47<88	0.11-0.20

[a]Derived by dividing the Maximum Acceptable Toxicant Concentration limits by the 96-h LC50.

Experiments at, or supported by, ERL,GB have demonstrated that sheepshead minnows are suitable saltwater fish for life-cycle toxicity tests. Because no other estuarine fish has been used in toxicant exposures continuing from reproduction through the growth of progeny, the usefulness of sheepshead minnows for determining application factors for other estuarine fish has yet to be evaluated. Results of experiments with diazinon, endrin, heptachlor, malathion, and trifluralin demonstrated striking similarities between application factors for sheepshead minnows and freshwater fish exposed to these chemicals. These data suggest that application factors for freshwater and saltwater fish are similar.

Physiology

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Concentrations of pollutants lethal to marine life are more easily determined than concentrations that have long-term effects on an aquatic animal's life span, growth, or movements. A prime objective of ERL,GB research is to develop new methods for evaluating subtle, sublethal effects of contaminants on the marine environment.

In 1977, ERL,GB investigators successfully used a tiny, shrimp-like crustacean (Mysidopsis bahia) throughout its life cycle (14 to 17 days) in toxicological and physiological studies during sensitive stages of development. Commonly called opossum shrimp (the female carries young in a brood pouch), mysids are an important element of estuarine plankton and an integral in estuarine and marine food webs (Fig. 4).

The species was first reported from West Bay, Galveston, Texas, and also has been observed in South Florida. The animals feed on 48-h-old brine shrimp (Artemia salina); their culture and maintenance in the test aquaria demands minimal time and effort. However, as required in the unautomated culture of most laboratory animals, continuous monitoring is necessary.

In 1977, 96-h toxicity tests and life-cycle tests with mysids demonstrated a sensitivity to cadmium and eight pesticides that was equal to or greater than the degree of sensitivity displayed by other estuarine biota (Table 4). Indicators monitored for effects in life-cycle tests include: susceptibility on the basis of sex, time required for formation of brood pouch, time required for release of brood, number of young released per female, survival of young to the F_1 and F_2 generations, and growth rate.

Table 4. Results of toxicity tests with Mysidopsis bahia

Compound	96-h LC50[1]	MATC[1]	AF
Cadmium	15.5	4.8 - 6.4	0.31 - 0.41
Diazinon®	4.83 (Adults)	1.15 - 4.4	0.24 - 0.91
Dimilin®	2.06	<<0.4[2]	<<0.19[2]
EPN	3.44 (Adults)	0.44 - 3.44	0.13 - 1.0
Kepone®	10.1	0.026 - 0.39	0.003 - 0.04
Leptophos	3.16	0.64 - 1.77	0.20 - 0.56
Methyl Parathion	0.78 (Juv.)	--[3]	--
Sevin®	7.7	2.8 - 7.7	0.36 - 1.0
Toxaphene	3.19 (Adults) 6.32 (Juv.)	0.067 - 0.14	0.02 - 0.04

[1] Micrograms per liter (µg/ℓ)
[2] Effects on reproduction were observed at an estimated 0.025 µg/ℓ.
[3] In progress

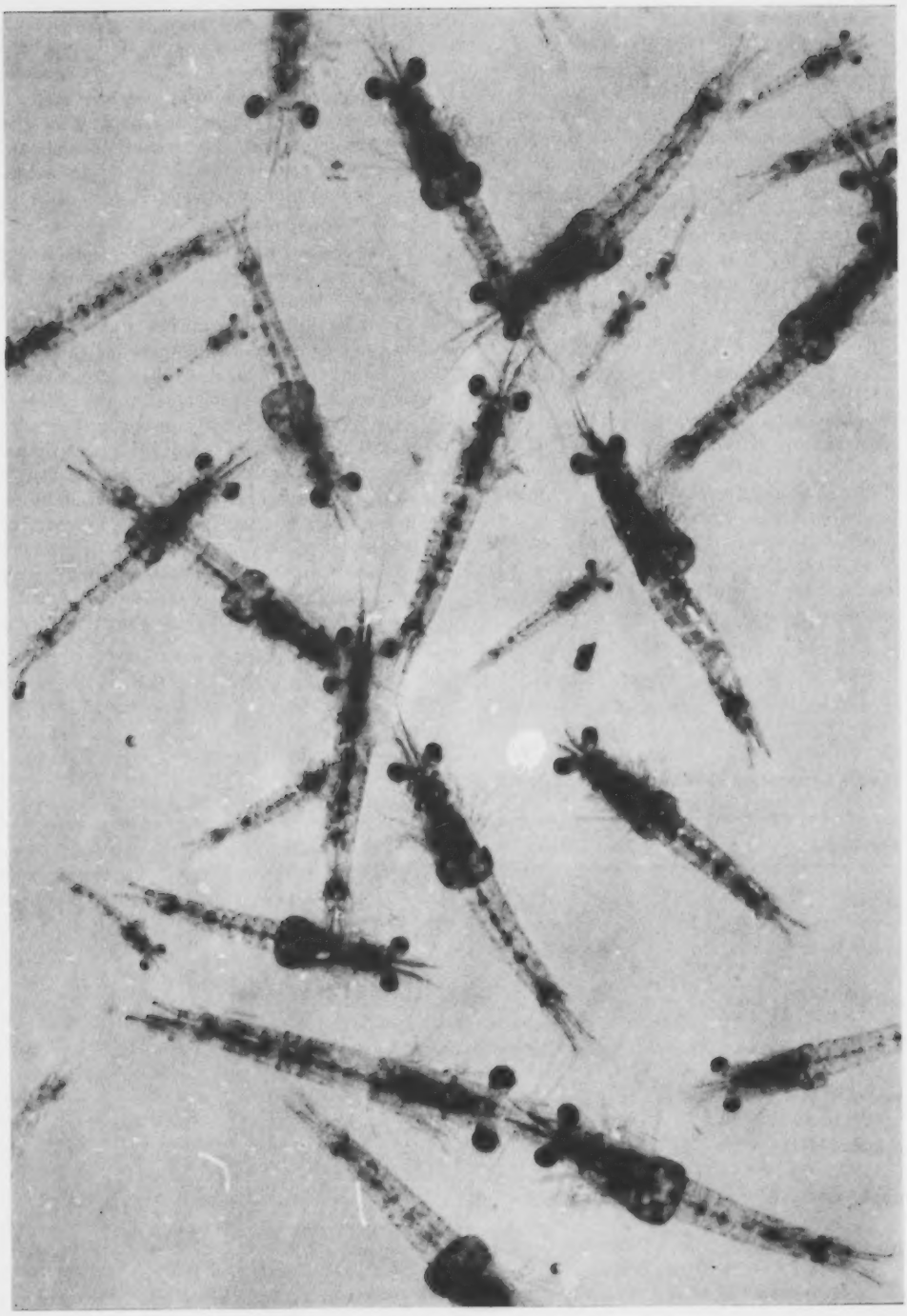

Figure 4. Mysid shrimp, 7 to 8 mm long, are cultured at ERL,GB in 38-ℓ glass aquaria containing filtered (20μ) flowing water (salinity = 10 to 27 parts per thousand, ppt). The species can be cultured continuously without fluctuations in population density on a diet of 48-h-old Artemia salina larvae

Table 5. Effect of Dimilin[R] on reproductive success of Mysidopsis bahia at 22-26°C and 22-28 parts per thousand salinity

Nominal Test Concentrations	µg/ℓ					
	Seawater Control	TEG* Control	0.075	0.25	0.50	0.75
Measured Test Concentrations	ND**	ND**	ND**	ND**	0.55	0.91
Total females	16	26	22	19	19	15
Juveniles produced	343	547	298	193	136	36
Juveniles per female, $\bar{X}$	21.4	21.0	13.5***	10.2***	7.2***	2.4***

*Triethylene glycol control
**Not detected; detection limit = 0.4 µg/ℓ.
***Significantly different from controls at = 0.05 (Dunnett's test).

Dimilin, a new insecticide that inhibits chitin synthesis in insects, was tested for the first time at ERL,GB in 1977 and found to be acutely and chronically toxic to mysids. During life-cycle studies, exposure to Dimilin affected the number of young produced by female mysids: only 13.5 young/female were produced in an estimated concentration of 0.075 µg/ℓ, whereas 21.4 and 21.0 were produced in controls (Table 5). As concentrations of Dimilin were increased, direct suppression of reproduction followed.

Tests were also conducted with toxaphene to determine life-long survival rates of young produced by exposed females. Parental mysids (24-h-old at outset) were exposed to toxaphene continuously until progeny (F_1s) were produced. Survival of young produced by parents exposed to 0.14 and 0.39 µg/ℓ toxaphene was equal to that recorded for control mysids; however, reproduction by young of exposed parents was either completely inhibited or drastically reduced. On the basis of reproduction as a criterion, investigators estimated that 0.067 µg/ℓ is a no-effect concentration of toxaphene (Table 6).

Table 6. Effects of toxaphene on survival and reproduction of mysid shrimp after a 14-day exposure followed by a 6-day depuration

Average Measured Toxaphene Concentration µg/ℓ	Survival (%)	Per Female
Seawater Control	81	6.8
Triethylene Glycol Control	69	7.8
0.14*	88	1.2**
0.39	67	0
1.3	19	0
4.2	0	0

*Temperature range, 20° to 26°C; salinity, 20 to 26 ppt
**Additional testing showed no significant decrease in number of young per female in an estimated 0.067 µg/ℓ toxaphene.

Table 7. Concentrations (µg/ℓ) of toxaphene acutely and chronically toxic to Mysidopsis bahia adults and juveniles

Days	LC50 (µg/ℓ)*			
	Juveniles	95% Fiducial Limits	Adults	95% Fiducial Limits
4	6.32	--	3.19	2.41 - 4.38
14**	0.70	0.38 - 1.47	0.85	0.61 - 1.21
20	0.66	0.32 - 1.58	0.59	0.37 - 0.99

Temperature range, 20 to 26°C; salinity, 20-26 ppt.
*Estimated by probit analysis
**Exposure halted

In the toxaphene tests, this pesticide was found to be slightly more toxic to adult mysids than to juveniles exposed for 96 h. However, continued exposure to toxaphene for 14 days, followed by 6 days without exposure, did not cause any apparent difference in susceptibility (Table 7).

In addition, mysids were used in a 96-h static test, followed by a 10-day study of reproduction success, to determine toxicity of a chemically contaminated sewage. The mysid shrimp was cultured at ERL,GB for transport by air to a mobile bioassay unit conducting field surveys of industrial waste in EPA Region IV (Southeast). Various laboratories, government and private, have reared mysids from stock obtained from ERL,GB.

Bioassay (Crustaceans)

DANA BETH TYLER-SCHROEDER, Research Biologist

Marine toxicologists have demonstrated that crustaceans are often more sensitive to organic pollutants than many other marine and estuarine organisms. However, few toxicity studies have been conducted with larval stages of shrimp because laboratory attempts to induce reproduction have been generally unsuccessful.

In 1977, experiments at ERL,GB demonstrated the usefulness of the grass shrimp (Palaemonetes pugio Holthuis) to monitor sublethal effects of a pollutant. These tests showed that grass shrimp are easily cultured in the laboratory, sensitive to toxicants, and can be maintained in flowing seawater systems for toxicity tests throughout a life cycle. Spawning was induced by temperature/light controls.

Initial tests with grass shrimp at ERL,GB determined the toxicity of endrin and its effects on shrimp development. Test animals were exposed to varying concentrations of endrin: 0.0 (control), 0.03, 0.05, 0.11, 0.18, 0.38, and 0.79 µg/ℓ in seawater.

Juveniles reached sexual maturity during the first two weeks of the exposure. Larvae spawned by control (unexposed) and exposed parents were continuously exposed until the juvenile stage (7 to 20 mm, rostrum-telson length).

Ability of the grass shrimp (Palaemonetes pugio) to complete life-cycle functions was seriously impaired by exposure to concentrations of endrin significantly lower than the 96-h LC50 (Table 7). The 96-h LC50 for exposed juvenile grass shrimp was 0.35 µg/ℓ. In the life-cycle toxicity test, gonadal development and spawning were inhibited at 0.03 µg/ℓ. Effects on reproduction were observed in the life-cycle toxicity test at all exposure concentrations tested. Effects on survival, larval development, and growth were observed at life-cycle test concentrations.

Survival of the parental generation was greatly affected after 2 weeks exposure to endrin concentrations of 0.38 µg/ℓ and above. At test termination, survival of parental shrimp was less than that in controls at concentrations of 0.11 µg/ℓ and above.

The onset and duration of spawning were significantly delayed and lengthened for female grass shrimp at all exposure concentrations (Fig. 5). Egg production and hatching success apparently were not affected at concentrations tested.

Larval mortality, length of time to metamorphosis, and growth of juvenile shrimp were impaired by endrin concentrations of 0.11 µg/ℓ and higher. Final length (rostrum-telson) of juvenile shrimp exposed to concentrations of 0.11 µg/ℓ and above was 12 to 65% shorter than control shrimp. Final weight of juvenile shrimp exposed to concentrations of 0.05 µg/ℓ and above was 26 to 94% less than control shrimp. However, growth of parental shrimp was unaffected.

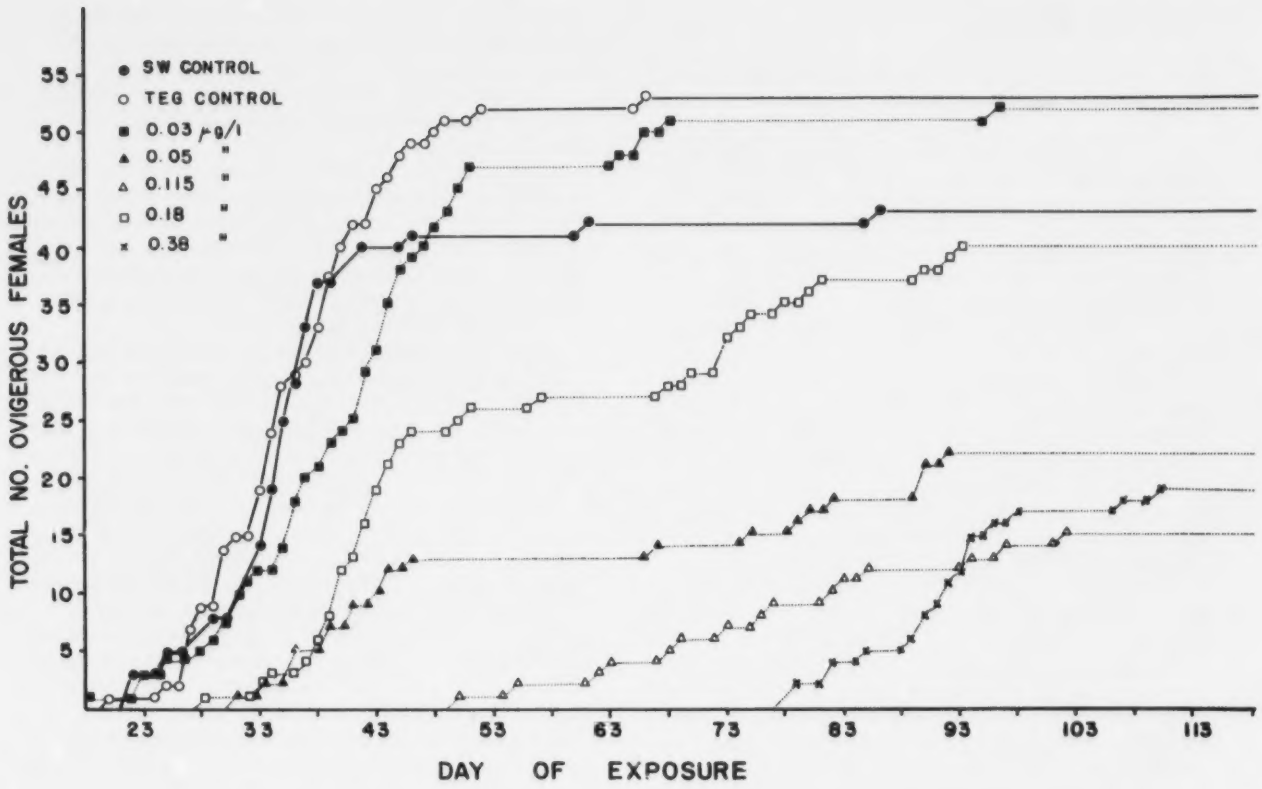

Figure 5. Effects on reproductive success of grass shrimp exposed to several concentrations of endrin in a life-cycle toxicity test. Exposures began with 100 shrimp per test concentration; the natural sex ratio for P. pugio is 50% female, 50% male.

In addition to the life-cycle tests with grass shrimp, ERL,GB staff in 1977 determined the acute 96-h LC50 of endrin-exposed first-day larval, juvenile, and adult grass shrimp and the rate of shrimp uptake and depuration of endrin. The specific application factor for grass shrimp was found to be less than 0.08, approximately one order of magnitude lower than for saltwater and freshwater fish (Table 8). These data were used by EPA to establish water quality criteria for endrin.

Table 8. Comparison of application factors generated for endrin

Species	Habitat	Concentration, µg/ℓ 96-h LC50	Concentration, µg/ℓ MATC	Application Factor
Cyprinodon variegatus	Saltwater	0.34	>0.12<0.31	0.35
Jordanella floridae	Freshwater	0.86	>0.22<0.3	0.25
Palaemonetes pugio	Saltwater	0.35	> ? <0.08	0.08

Community Bioassays

MARLIN TAGATZ, Research Aquatic Biologist;
JOEL IVEY, Biological Technician

Community bioassays offer another facet to scientific analyses of the environmental impact of man-produced pollutants. These tests enable investigators to determine simultaneously the relative sensitivity to toxicants of many different types of organisms (more than 100 species of 9 phyla have been collected). Animals are exposed to test compounds in their early development stages, rather than in their later, and often more resistent, juvenile or adult stages.

Community bioassays conducted in 1977 at ERL,GB sought to determine effects of oil drilling fluids on the development of estuarine communities. Planktonic larvae in flowing seawater from Santa Rosa Sound were allowed to colonize in a group of aquaria. After about 10 weeks, the number and species of animals (marcoinvertebrates) exposed to various concentrations of the test fluids were compared statistically to those in unexposed aquaria.

Four experiments with drilling muds were completed during the reporting period. Pentachlorophenol and sodium pentachlorophenate, used to control bacteria in drilling muds, altered development of exposed estuarine communities. Total numbers of individuals and species were significantly fewer in aquaria exposed to 76 µg/ℓ (parts per billion) pentachlorophenol or 161 µg/ℓ sodium pentachlorophenate than in unexposed aquaria. As little as 15.8 µg/ℓ sodium pentachlorophenate reduced the total number of animals but not the total number of species. Molluscs, arthropods, and annelids were particularly affected by these compounds.

Barite ($BaSO_4$, weighting agent and primary component of drilling muds) and a lignosulfonate type of whole mud (obtained from a drilling operation) also affected the composition of estuarine communities. Significantly fewer animals and species developed in aquaria sand covered by 5 mm of barite or 2 mm of the mud than in unexposed aquaria. Settling organisms were also affected when these toxicants were mixed with the sand. A ratio of 1 part barite to 3 parts sand, or a ratio of 1 part drilling mud to 10 parts sand, significantly reduced the total number of animals. Annelids and molluscs were the most sensitive to barite; annelids and coelenterates were the most sensitive to whole drilling muds.

Results of ERL,GB community bioassays will be compared with concurrent field studies on a platform in the Gulf of Mexico. Future tests of drilling muds, pesticides, and other toxic organics should provide additional parameters of toxicity and more data for broader ecological application.

Food Chain Studies

LOWELL BAHNER, Research Aquatic Biologist

The uptake of pesticides by estuarine animals through bioconcentration from water or bioaccumulation from food is a useful indicator of pesticide movement in the natural environment. Field surveys indicate that food is a prime source of the pesticide contamination of certain estuarine species.

Food Chain Studies at ERL,GB seek to evaluate the magnitude of pesticide transfer to estuarine animals via water, food, or bottom sediment. Results of these studies are used in (1) interpreting field test data; (2) predicting sublethal effects of pesticides on the natural environment; (3) developing ecosystem models to predict long-range movements of pesticides in ecosystems.

Tests in 1977 exposed 10 species of estuarine organisms to toxaphene, trifluralin, Kepone, diazinon, and Halowax^{R} 1014 in water, food, or sediments. Results indicate that: (1) toxaphene, trifluralin, and Halowax 1014 accumulate in tissues of grass shrimp and spot, and (2) trifluralin will transfer to spot when fed copepods contaminated with the chemical. Grass shrimp exposed for 28 days to 0.1 or 10.0 µg/ℓ diazinon contained no detectable diazinon residues, but all shrimp exposed to 10.0 µg/ℓ died by day 15 of exposure. Sheepshead minnows or pinfish that fed on grass shrimp or mysids exposed to diazinon had no measurable diazinon residues, but cholinesterase activity in the fish was suppressed by 15%, compared to control fish fed uncontaminated living shrimp or mysids.

Six species--oysters, grass shrimp, polychaete worms, fiddler crabs, blue crabs, and spot--were exposed to James River sediments containing 0.2 to 0.4 µg Kepone/g. The fiddler crabs and polychaete worms ingested the fine sediments as indicated by residues of 0.3 µg/g in whole-body samples. The other four species contained residues comparable to concentrations of Kepone in animals that received Kepone (0.02 µg/g) from water only. Results indicated that field-exposed animals (with residues greater than water-exposed animals) obtain significant portions of Kepone from food.

Additional laboratory food chains will be developed by testing and culturing alternate food chain organisms. These organisms will be used to determine if quantity and quality of various foods affect pesticide availability to predatory fish.

Algal and Protozoan Studies

NELSON R. COOLEY, Microbiologist

Protozoa, algae, and bacteria form the broad base of aquatic food chains. Ciliated protozoa are the most numerous organisms of the estuarine benthos and may be more important than bacteria as nutrient regenerators, particularly of nitrogen and phosphorus.

Further, some ciliates are able to concentrate certain persistent pesticides and thereby aid in translocating them. Thus, effects of these toxicants possibly could be exerted at higher trophic levels either through the disruption of nutrient cycles or through their translocation and biological concentration in the food chain.

Algal and protozoan studies at ERL,GB in 1977 examined the effects of toxicants on population growth of marine unicellular algae and ciliate protozoans and the toxicant bioaccumulation/bioconcentration by test organisms.

The ERL,GB investigation evaluated changes in biomass of algae due to toxicants on the basis of changes in optical density (OD). (OD values are relative; i.e., they are not absolute numbers of cells or of volume of cellular material per unit volume of culture.)

In addition, changes in cell counts/ml (determined optically or electronically) were used to measure population changes. In certain instances, cell numbers can decrease while cell volumes simultaneously increase, thus causing no significant change in biomass.

Population growth of two species of algae (10 randomly chosen cultures of each species) was measured daily for 10 days. The OD of each culture was read on a photometer, then cell counts and cell volumes were determined with a Coulter ZBI Counter and P-64 Size Distribution Analyzer. Data were subjected to linear regression analysis and ANOVA (analysis of variation).

For Dunaliella tertiolecta, mean cell volume per ml was linearly correlated with mean OD ($r = 0.941$), the F-value for regression being 761.065 ($\alpha < 0.001$), and with mean cell volume ($r = 0.959$), the F-value for regression being 1117.885 ($\alpha < 0.001$). For Chlorococcum sp., similar results were obtained. One other species of different body form will be tested before this study is concluded.

Studies of (1) interaction of 2,4-D and metals with and (2) effect of Sevin on population growth of marine unicellular algae were performed in cooperation with Dr. Gerald Walsh; results are reported on p. 14.

The ciliate protozoan (Tetrahymena pyriformis) was exposed in flask cultures at 26°C to chrysene, a petroleum-related polynuclear aromatic hydrocarbon. Test concentrations that ranged in 10-fold steps from 0.0024 to 2.4 parts per million (ppm) exerted no significant effect ($\alpha = 0.05$) on either 24-h growth rate or on 96-h population size.

However, in a 24-h test, this ciliate bioaccumulated and bioconcentrated chrysene 97 times the initial concentration in the medium (from 1 ppm in the medium to 97 ppm in the cells). In 24-h and in a test lasting 120-h, it bioaccumulated and bioconcentrated chrysene 144 times the initial concentration in the medium (from 5 ppm in the medium to 722 ppm in the cells). Similar bioaccumulation/bioconcentration in nature would permit the chemical's entry into aquatic food chains. Test results indicate that chrysene might serve as a useful model to determine how other polynuclear aromatic hydrocarbons are bioaccumulated and bioconcentrated.

In earlier continuous-flow tests at ERL,GB, a simple homemade chemostat was used to study the effect of chrysene on cultures of T. pyriformis at room temperature. Although bioconcentration of chrysene by the ciliate could be demonstrated, growth data were equivocal because of the many uncontrolled environmental variables inherent in this chemostat. A new commercially made chemostat was put into operation in 1977 to control medium flow-rate, rates of agitation and aeration, and temperature, as well as record pH and dissolved oxygen. A simple device was also added for aseptic delivery of measured amounts of sterile Antifoam B to prevent foaming of the medium. This system will be used to develop a continuous-flow bioassay for toxicants and possibly to study the fate of the toxicants and to supply contaminated organisms for food chain studies.

Toxicity of the carbamate insecticide, Carbaryl (Sevin^{R}) to Tetrahymena Pyriformis W (grown axenically in Tetrahymena medium) also was studied in 1977. Test concentrations of Carbaryl ranged from 0.001 to 30 ppm. Greatest observed decrease in 24-h growth rates occurred at 30 ppm, but were only 7.0% to 10.5% less than control growth rates. Reductions of population sizes at 96 h when exposed to 30 ppm did not exceed 12.0%. Liquid chromatographic analyses of cells grown for seven days in the presence of Carbaryl revealed no significant bioconcentration of Carbaryl, but considerable 1-naphthol was recovered. The latter compound is a breakdown product of Carbaryl.

Effects of Industrial Wastes on Selected Estuarine Flora and Fauna

G.E. WALSH, Research Biologist

A mobile laboratory was established by ERL,GB in 1977 to conduct "at-the-pipe" field surveys of industrial waste disposal sites. The mobile bioassay unit provides data supplemental to ERL,GB laboratory analyses and required by the Surveillance and Analysis Division of EPA's Region IV.

A trailer containing 125 square feet of floor space serves as the mobile laboratory (Fig. 6). It is equipped with two proportional diluters for bioassays with mysid shrimp and juvenile sheepshead minnows and with instruments for monitoring pH, Eh, temperature, salinity, and dissolved oxygen.

Bioassays of wastes from seven industrial plants (producing textiles, paper, and chemicals) were completed during the reporting period. Field analyses and algal bioassays at ERL,GB of samples showed that both algal and animal tests are essential for a complete analysis of the biological effects of wastes. For example, in ERL,GB tests, certain effluents did not affect survival of fish or mysid shrimp, but caused algae to grow rapidly and attain high population densities.

In conjunction with the investigation of industrial wastes, a new method for a rapid screening algal bioassay was developed at ERL,GB and was successfully tested with waste samples from 16 plants associated with the textile industry. The method allows Skeletonema costatum grown in optically matched tubes to be measured by spectrophotometry up to 96 h. In general, algal tests were more sensitive than comparable tests in which animal

Figure 6. Dr. Alan Auwarter examines aquaria in ERL,GB's mobile bioassay laboratory used in field surveys of industrial effluents.

methods were used. Some wastes were found to contain high concentrations of plant nutrients.

Bioassays of industrial plants with the mobile laboratory will be continued in 1978, and laboratory studies on effects of complex wastes will be initiated on phytoplankton communities.

Effects of Pesticides on Population Growth of Marine Unicellular Algae

GERALD WALSH, Research Biologist

Effects of Sevin, 2,4 - D + nickel, 2,4 - D + aluminum, Kepone, and Leptophos on marine unicellular algae were studied in 1977.

Sevin was not highly toxic to Chlorella sp., Chlorococcum sp., Nitzschia sp., or Skeletonema costatum. The average EC50s, in ppm, were: Chlorella, 0.9; Chlorococcum, 23; Nitzschia, 1.0; and Skeletonema, 1.5. Uptake of Sevin or its metabolite, 1.napthol, was not found when Skeletonema was exposed to 0.05 ppm for 24 h. Future work will be limited to studies on chemicals that require a rapid assessment.

The Dynamics of an Estuary as a Natural Ecosystem

F.J. VERNBERG, Principal Investigator, EPA R804407, Univ. of South Carolina, Columbia, S.C.; G.E. WALSH, Project Officer

In this study, investigators attempted to (1) establish data on an undisturbed estuary for comparative studies on effects of various stresses of pollutants on other estuarine environments; and (2) develop ecosystem models for predicting probable effects of environmental perturbation. The principal objective of a microecosystem study was to develop and test replicate experimental units to be used to assess long- and short-term effects of pollutants on the Spartina alterniflora salt-marsh community.

An interdisciplinary team of marine scientists developed a conceptual model of energy flow for a marsh-estuarine ecosystem, using three subsystems (water column, intertidal marsh zone, and benthic subtidal zone). A linear dynamics system with 22 states was chosen as the mathematical model.

In addition, investigators developed a linear systems model of the intertidal oyster community. Results were reported in the EPA Ecological Research Series, EPA-600/3-77-016, January 1977.

Water Quality and Mangrove Ecosystem Dynamics

S.C. SNEDAKER, Principal Investigator, EPA R804355, University of Miami, Miami; G.E. WALSH, Project Officer

A report on results of work will be published in 1978. The project will provide data on biomass of several compartments of estuarine forests, their turnover and productivity rates, amount of selected pesticides in the compartments, and the rate of pesticide exchange and loss.

Effects of Selected Wastewater Chlorination Products and Captain on Marine Algae

H.C. SIKKA, Principal Investigator, EPA Grant R803943, Syracuse Research Corporation, Syracuse, NY; G.E. WALSH, Project Officer

The release of potentially toxic chlorinated organics in the aquatic environment is an environmental concern. Further understanding of the effects and fate of chlorination products in the biota is required to evaluate the impact of waste-water chlorination. This research grant examined effects of stable organic compounds produced during chlorination of sewage effluents and the pesticide captan on phytoplankton, which contribute oxygen to the aquatic environment.

Test results showed that:

. 3-Chlorobenzoic acid (1 or 10 ppm) had either no or a slight effect on growth of Dunaliella or Porphyridium. It inhibited growth of Skeletonema at 10 ppm, but had no marked effect at 1 ppm.

. 5-Chlorouracil at 1 or 10 ppm did not affect Skeletonema, but stimulated growth of Dunaliella initially.

. 4-Chlororesorcinol had no effect on Dunaliella at 1 ppm, but 10 ppm of the chemical caused a small decrease in growth. The chemical produced an initial stimulation in growth of Porphyridium, followed by an inhibition. Growth of Skeletonema was inhibited by 4-chlororesorcinol at concentrations ranging from 1 to 10 ppm.

. 3-Chlorophenol stimulated growth of Dunaliella. Skeletonema growth was inhibited at concentrations higher than 2.5 ppm, but showed some stimulation at 1 ppm. At 1 ppm it stimulated growth of Porphyridium, but was slightly inhibitory at 5 ppm.

. A combination of 3-chlorophenol and 4-chlororesorcinol interacted synergistically to reduce Skeletonema growth.

. Captan suppressed growth of Dunaliella and Porphyridium at a concentration of 5 ppm. Slight stimulation in growth of the two organisms was noticed in the presence of 0.1 and 1 ppm of the fungicide. Captan was inhibitory to Skeletonema at concentrations ranging from 0.25 to 5 ppm. Treatment of Skeletonema with 0.5 ppm of Captain for 30 min caused a substantial reduction in photosynthetic $^{14}CO_2$ fixation.

These findings are reported in EPA-600/3-77-029, Ecological Research Series, March 1977.

Acute Static Bioassay

PATRICK W. BORTHWICK, Research Biologist

Acute static bioassays (≤96 h) are used to rapidly assess the immediate environmental hazards of toxic compounds to single marine species. Static tests effectively measure toxicity of little-known substances and contaminants available in limited qualities.

In 1977, these bioassays were performed at ERL,GB to evaluate hazards of complex wastes, such as industrial effluents, sewage treatment plant outfalls, and materials dumped in the ocean.

In response to an emergency request from officials in Louisville, Kentucky, ERL,GB tested sludge from the city's main sewage treatment plant to determine if its disposal in the ocean would threaten marine life. The Louisville treatment plant was contaminated in April 1977 by a substantial quantity of hexachlorocyclopentadiene. The treatment plant was forced to cease operation until the toxic sludge could be removed.

ERL,GB static bioassays showed that the sludge was toxic to marine life (Table 9) and that the poisonous substance would bioaccumulate in the aquatic food web. These findings were corroborated by marine copepod toxicity tests conducted by Bionomics Marine Research Laboratory in Pensacola and by tests with marine diatoms conducted by Dr. G.E. Walsh at ERL,GB. Test results lead to a decision for on-land disposal of the sludge.

In another series of tests, ERL,GB conducted bioassays on undiluted effluents from four ocean outfalls in response to a request by EPA's Office of Water Programs. Tests showed that none of the effluents were toxic to sheepshead minnow fry.

Future studies will attempt to develop static toxicological methods to determine hazards of industrial wastes to fish and macroinvertebrates.

Table 9. Acute Toxicity of Louisville Sludge

TEST ORGANISM	PERCENTAGE LOUISVILLE SLUDGE BY SERIAL DILUTION 96-h LC50	(95% CONFIDENCE INTERVAL)
Marine copepods	0.0025	(0.0013–0.0046)
Mysid shrimp	0.047	(0.024–0.100)
Grass shrimp	0.073	(0.054–0.105)
Sheepshead minnows	0.32	(by interpolation)
Marine diatoms	*0.068	(0.052–0.088)

*96-h EC50

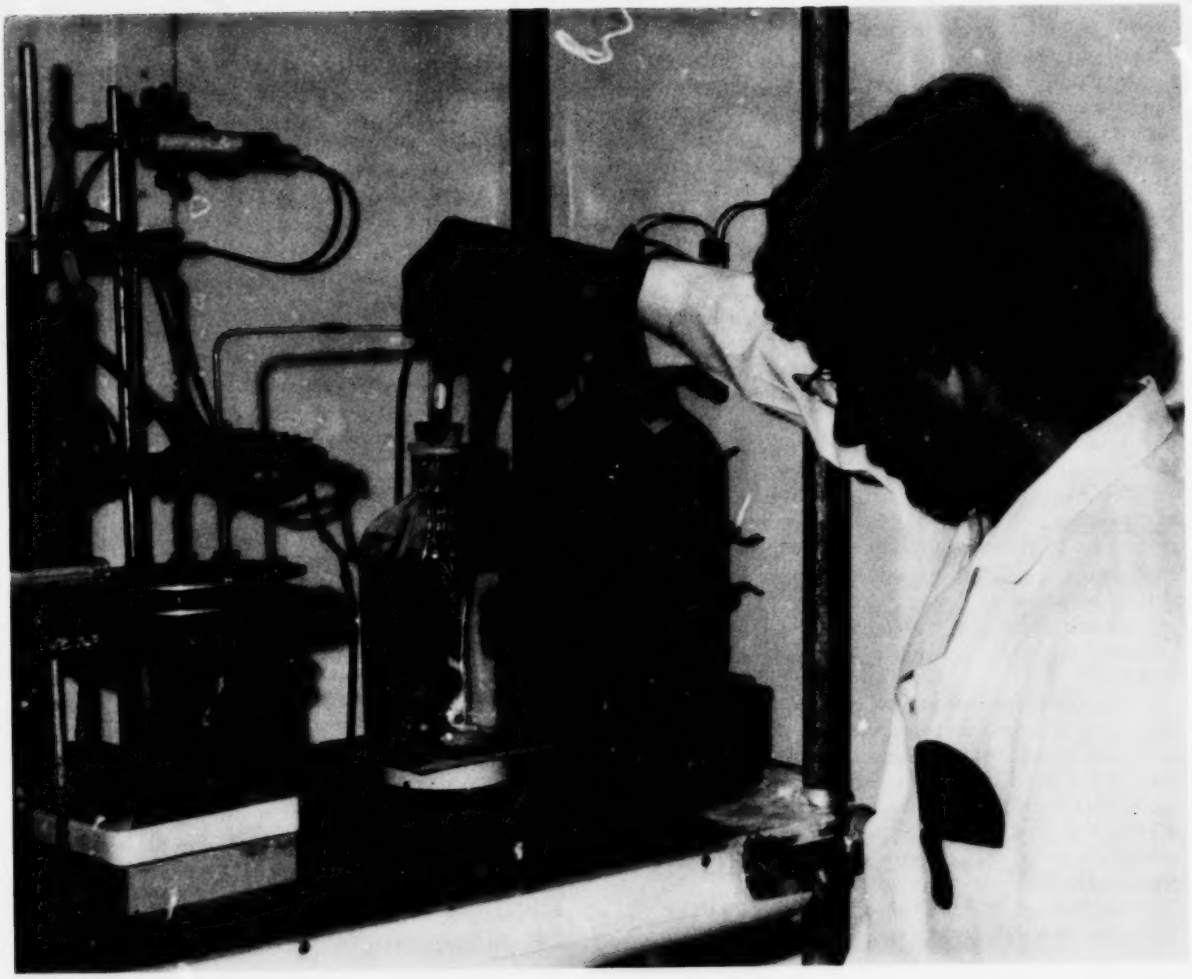

Figure 8. Dr. H.P. Pritchard examines a continuous-flow system developed at ERL,GB to monitor how a pollutant moves and is transformed in an estuarine environment.

PROCESSES AND EFFECTS BRANCH

FRANK G. WILKES, Chief

The development and validation of a Hazard Assessment Model to predict risks caused by toxicants entering the marine environment are primary goals of EPA's Office of Research and Development. Two major components comprise the model: (1) the toxicity component that describes effects of specific pollutants at given concentrations on organisms and ecosystems; (2) the exposure component that describes and predicts concentrations and duration of toxic exposures.

Research conducted by the Processes and Effects Branch in 1977 was directed toward the development of data for an Exposure Assessment Model capable of predicting toxicant concentrations released in marine ecosystems. The model will predict modifications of toxicant concentrations brought about by biological, chemical, and physical interactions with a chemical and its by-products. This model eventually will be linked or combined with other models that predict toxicity of pollutants after differing duration and exposure concentrations.

Data used in the development of the Exposure Assessment Model are derived from tests using "microcosms" or miniature ecosystems supported by life processes. Microcosms represent segments of the environment that can be monitored to evaluate integral interactions among marine organisms and alterations of their physical/chemical surroundings.

Another objective of the Processes and Effects Branch is to develop new methods for evaluating changes in ecosystem compartments subjected to stress caused by hazardous organic or inorganic pollutants. In 1977, branch scientists worked on a broad spectrum of tests varying in complexity and purpose (Fig. 9). Selection of a test or combination of tests is determined by the pollutant, the aquatic environment under study, and the intended use of the data generated by the test. Microcosms also provide data related to Water Quality Criteria and analyses of the environmental risk of toxic substances.

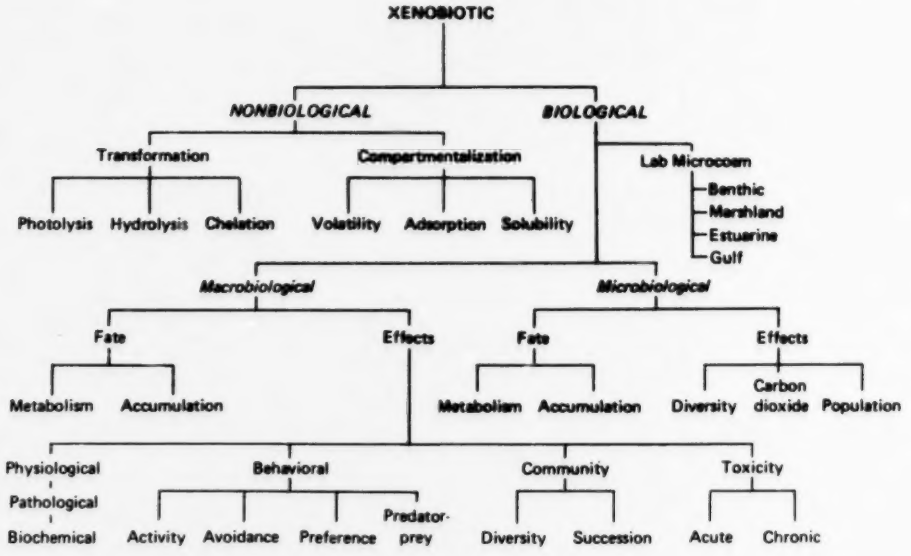

Figure 9. Tests in diagram are used to determine the effects of xenobiotics on ecosystem processes and components.

Microcosms

The impact of pollutants on estuarine environments can be assessed in reproducible laboratory systems ("microcosms") that isolate the physical or biological components under study.

Tests under development in the Processes and Effects Branch range from systems that investigate the fate of pollutants in an estuarine environment to a system that focuses on how pollutants affect specific behavioral responses. During 1977, ERL,GB scientists studied the fate of a pollutant to determine its transport route, availability, and its transformation. For example, the insecticide methyl parathion was found to degrade into a number of chemicals demonstrating different toxicities and characteristics (Fig. 10).

Investigators developed the following systems, with methyl parathion as the pollutant:

. Environmental Fate Screening System. The effects of a pollutant are closely related to its availability and chemical form. This system allowed investigators to determine the environmental processes and compartments that influence the movement and transformation of pollutants.

. Eco-core System. This technique isolated indigenous microorganisms from intact environmental sediment-water cores and evaluated their potential to degrade pollutants.

. Continuous-flow Systems. These large- and small-scale systems incorporate flowing water and allowed investigation of dynamic environmental processes.

. Aquatic Gradient Avoidance Response System (AGARS). This system used behavior as an indicator of chemical exposure and allowed quantification of the avoidance responses of estuarine organisms to different pollutant concentrations.

. Benthic Bioassay System. Changes in sediment surface features produced by lugworms (Arenicola cristata) exposed to toxicants were used to monitor effects of pollutants on a benthic infaunal organism.

Environmental Fate Screening System

R.L. GARNAS, Investigator

The Environmental Fate Screening System was designed to determine ecosystem substrate exchange coefficients

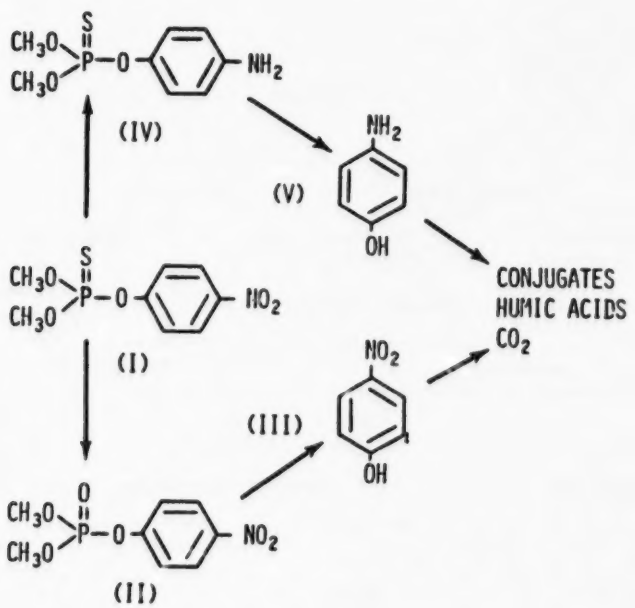

Figure 10. Projected pathway for the breakdown of methyl parathion (I) in aquatic systems. Compounds are: methyl paraoxon (II), p-nitrophenol (III), amino methyl parathion (IV), and p-aminophenol (V).

and rates of pollutant alteration. Environmental factors that influence pollutant fate are: sediment type (sorption processes: clay, organic), water quality (salinity, Eh, pH), physical forces (temperature, sunlight, volatilization), and biological factors (microbiological processes: metabolism, degradation).

In the analysis for the pollutant, the system is fractionated into water, suspendible particulate, and heavier, nonsuspendible sediment or sand. These fractions are extracted separately with an organic solvent and analyzed by thin layer chromatography/autoradiography, with confirmational analysis by gas or liquid chromatography. The extracted sand and particulate fractions are combusted at 900°C to liberate $^{14}CO_2$ to determine unextractable (bound) residues. All exit routes and system components are sampled; therefore, a materials balance can be calculated.

In 1977, ERL,GB investigators used sterile and nonsterile systems to study the movement of the insecticide methyl parathion in a simulated salt-marsh environment (Table 10). The water compartment (W) in the non-sterile systems demonstrated rapid degradation to amino methyl parathion and unextractable products. After 14 days, the parent compound was undetectable. The radioactivity in

Table 10. Methyl parathion: percentage[a] radioactivity distribution

Day	W	Se	Sc	Pe	Pc	R	Total
Non-sterile Systems							
0	67.3	0.9	0.1	22.6	5.6	0	96.5
4	30.7	0.7	2.2	6.5	48.5	0.3	88.9
7	28.2	0.5	1.1	5.4	55.4	0.2	90.6
11	27.4	0.4	1.8	3.9	55.2	0.3	89.0
14	20.5	0.3	2.3	2.9	63.7	0.1	89.8
18	18.8	0.2	2.3	2.7	64.2	0.3	88.5
21	19.4	0.3	2.4	2.2	64.5	0.3	89.1
25	18.3	0.3	1.6	2.5	63.9	0.3	86.9
Sterile Systems							
0	68.8	0.9	0.1	29.3	0.7	0	99.8
7	46.9	1.5	0.2	45.1	2.9	0.2	96.8
14	49.0	0.9	0.2	33.7	4.8	2.2	90.8
21	50.0	1.3	0.3	30.8	5.1	3.4	90.9

[a]100% = 8.8 g = 2,649,100 dpm

W - water

Se - nonsuspendible sediment extract

Sc - nonsuspendible sediment combustion

Pe - suspendible particulate extract

Pc - suspendible particulate combustion

R - volatilization

these systems moved quickly to the detrital particulate fraction (Pc) where it became irreversibly bound; volatilization (R) and interaction with nonsuspendible sediments (Se) were of minor importance in the fate of this chemical. Sterile systems displayed little transformation of methyl parathion; the chemical was either in the water column or extractable from the suspendible particulate (Pe). These data suggest that the irreversible binding of methyl parathion to the suspendible particulate fraction of a salt-marsh ecosystem is partly a result of microbial action. This phenomenon has been reported previously for agricultural soil and may be of general occurrence for many organophosphorus insecticides.

Due to the static nature and small size of this system, further validation of these results is required. The system, however, has proven to be a valuable screening tool because of its simplicity.

Eco-core System

A.W. BOURQUIN, Research Microbiologist

The main agents for returning organic carbon compounds to the carbon cycle are bacteria and fungi. These organisms affect mineralization of natural compounds and might be expected to degrade those of synthetic origin. Therefore, microbial considerations are essential in the response and recovery of natural and artificial ecosystems from the stress of chemical perturbations.

The Eco-core, an artificial microbial microcosm, closely mimics natural conditions for studying microbial interactions. The system evaluates the degradative potential of indigenous microorganisms and the effects of chemical perturbation on microbial ecology. The system utilizes sterile glass cylinders to extract intact sediment and water cores from the environment. Degradation products are continuously monitored in the water column by thin-layer chromatography and autoradiography.

Methyl parathion (MPS) fate and effects were examined in a salt-marsh Eco-core. Table 11 shows the disappearance of MPS from the water column for three types of cores: (1) seawater (water), (2) water and sediment (sediment), and (3) sterilized water and sediment (sterile). The sterile cores showed only a slight decrease of MPS (82% extractable after 30 days). All other cores showed rapid disappearance of MPS from the water column. Radioactivity in all sediment cores decreased little after 20 days incubation; however, analysis of the radioactivity indicated the presence of a major degradation product, aminomethyl parathion (AMPS). The decrease in MPS concentration in water cores was due primarily to adsorption to the glass in both sterile and non-sterile systems. Degradation of MPS was considerably less in water cores than in cores containing sediment.

Polar products (Table 11) represented the organic solvent-unextractable radioactivity in the water column and occurred to the greatest extent in the cores containing sediments. Production of $^{14}CO_2$, another indication of aromatic ring cleavage in the parent molecule (MPS), occurred to a greater extent in the sediment cores than in the cores with water only (Table 12). No significant $^{14}CO_2$ evolved from the sterile systems.

Total recovery of the radiolabel amounted to greater than 90% in all cases. Combustion of the extracted sediments to $^{14}CO_2$ showed 7% of the radioactivity to be unextractable from the sterile sediment systems. Results indicate sediments may be necessary for rapid metabolism of methyl parathion.

Table 11. Metabolism of methyl parathion in a salt-marsh Eco-core

	Percentage of MPS remaining[a]			Percentage of polar products		
Day	Sterile	Sediment	Water	Sterile	Sediment	Water
0	100	100	100	0	0	0
2	92	80	85	4	8	3
4	85	57	66	6	13	7
8	84	15	27	11	23	9
14	84	4	15	16	40	10
31	82	0.01	6	21	54	9

[a]MPS added = 1 mg/ℓ containing 22.2 μCi.

Table 12 Evolution of $^{14}CO_2$ from ^{14}C-methyl parathion

	Radioactivity (DPM x 10^2)		
Day	Sterile[a]	Water	Sediment
0	0	0	0
2	0	0	2
4	8	113	1520
8	3	133	910
14	5	380	830
24	4	716	1956
31	2	990	1550
34	2	554	789
% Total dpm added	0.02	3	6

[a]Water and sediment treated with 2% formalin

As a technique for assessing biodegradation of xenobiotics, the Eco-core system can: 1) determine interactive processes contributing to the metabolism of the test compound, 2) monitor changes in microbial populations induced by the toxicant, and 3) easily facilitate system replication. The core, however, is a static system with a disadvantage of possible artifactual changes that can be induced by accumulating metabolites.

Continuous-flow Systems

H.P. PRITCHARD, Environmental Scientist

Continuous-flow systems (Fig. 8, p. 16) provide information on the rate and extent to which a pollutant: a) moves into the various compartments of an aquatic ecosystem and b) is transformed by biological and non-biological forces.

Small-scale System -- A small-scale continuous-flow system (500-ml reactor vessel volume) was used to study the fate of methyl parathion under conditions typical of an aquatic estuarine environment. This system (Fig. 11) was designed with multiple stages to study transformation processes as a function of dilution. It used fresh seawater and artificial seawater, supplemented with radiolabeled methyl parathion (50-500 ppb). Transformation products were quantified by thin layer chromatography, autoradiography, and gas chromatography. Radio-labeled MPS was added to the system either as a spike into the primary reactor vessel (wash-out kinetics followed) or continuously from a reservoir (quasi-steady kinetics followed).

Table 13 shows the stability of MPS in sterile and unsterile continuous-flow systems without sediment. Very little chemical or biological degradation of the substrate occurred under varied conditions (differing flow rates, nutrient concentrations, seawater, and organic supplements). However, MPS was much less stable when sediment was added to the system. Aeration was adjusted to give an aerobic water column and an anaerobic sediment-water interface. Table 14 shows that MPS stability, as indicated by CO_2 and polar product production in the unsterile system, decreased with time. This presumedly reflects the enrichment of an MPS degrading population of bacteria. MPS was also transformed in the sterile control but to a lesser extent. Amino methyl parathion (AMPS) was the principle transformation product in both systems, but p-nitrophenol was also detected. The presence of sediment, therefore, promoted the transformation of MPS in estuarine seawater environments. Calculations from these initial experiments indicated a half-life of MPS of 400 to 500 hours for the conditions tested. If methyl parathion input to the continuous-flow system is eliminated (but seawater flow is maintained), residual MPS in the sediment (shown in the other systems to be tightly bound) is slowly released with time.

Large-scale Systems -- Large-scale, continuous-flow systems (40-ℓ reactor vessel volume) are designed to study

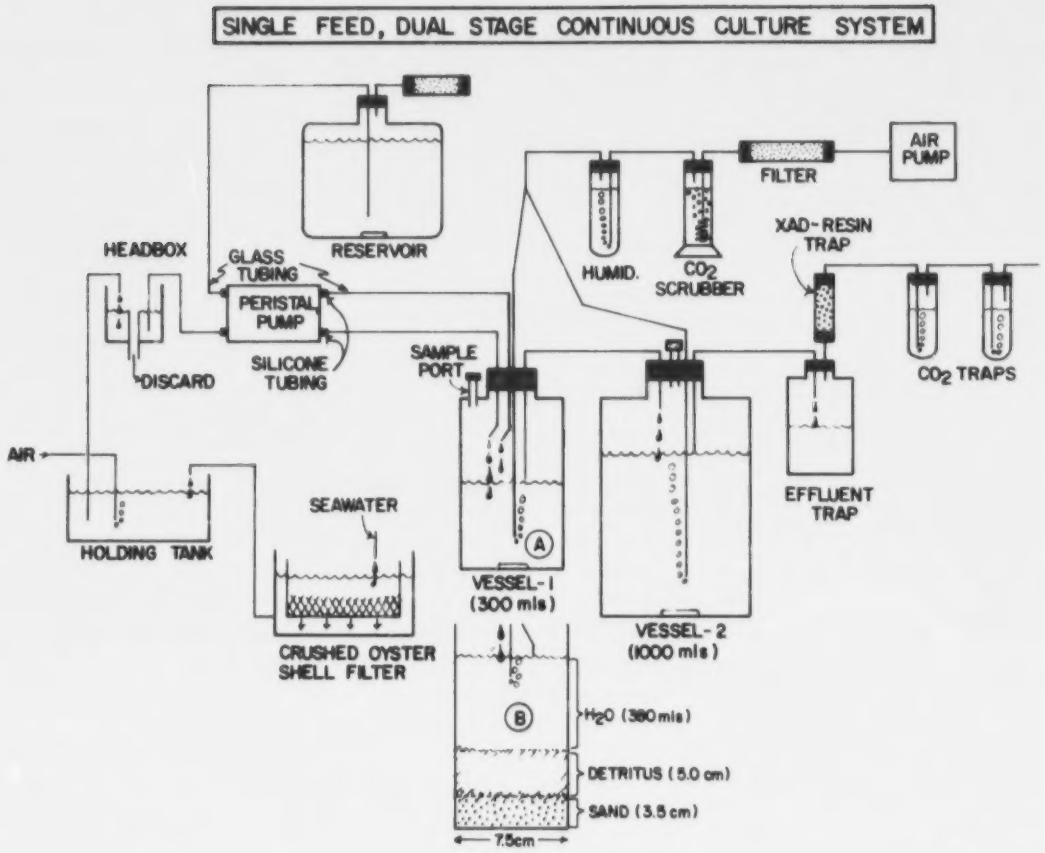

Figure 11. Schematic design for a continuous-flow microcosm. A and B represent two options for systems developed at ERL,GB to assess fate and transport of pesticides in estuarine environments.

the fate of a pollutant affected by marine macrobiota. Thirty liters of seawater were added to a vessel containing ^{14}C methyl parathion and a 9-cm layer of marsh sediment. The systems were continuously fed fresh filtered seawater and all water exited through polystyrene resins. Toxicant movement into the sediment was the major transport process.

Sediment-burrowing lugworms (Arenicola cristata) caused a more rapid dispersal of radioactivity throughout the sediment column of reactor vessels. These worms enhanced the movement of radioactivity from the water column to the sediment, but accumulated no radioactivity. Radioactivity budgets of the systems suggested accumulation of unextractable residues in the sediment, a process verified by the other systems discussed. Analysis of extractable radioactivity indicated both p-nitrophenol and amino-methyl parathion as degradation products. The amount of degradation was affected by microbial activity and not by lugworms.

The selective nature of continuous-flow systems accommodates the study of many complex biological processes separated into their component parts. Rates of transformation and compartmental exchange obtained from continuous-flow studies are considered more appropriate for environmental studies than similar rates derived from static tests.

Aquatic Gradient Avoidance Response System

R.L. CRIPE, Research Biologist. EPA Grant R804458, University of West Florida, Pensacola; C.N. D'ASARO, Principal Investigator; F.G. WILKES, Project Officer

Behavioral tests facilitate the study of subtle, sublethal effects of certain pollutants. Long-term ecological consequences of avoiding lethal or sublethal toxicants in an area near a pollutant source may affect the marine environment, either overtly (a kill) or through less obvious mortalities at the lower trophic level. Inability of aquatic organisms to avoid contaminants also may cause harmful substances to bioaccumulate in the aquatic food chain.

Most apparatus designed to detect avoidance of pollutants by aquatic organisms require visual observations of

Table 13. Stability of methyl parathion in continuous-flow systems without sediment.

Retention Volume[a]	Concentration of Methyl Parathion (mg/l)			
	Sterile Control		Unsterile System	
	Reservoir	Vessel	Reservoir	Vessel
1.3	1.10	0.97(81%)[b]	0.97	0.92(94%)
4.2	1.23	1.06(85%)	1.07	0.89(83%)
8.3	0.87	0.97(90%)	1.10	1.04(94%)
11.3	1.08	0.92(85%)	1.03	0.90(87%)
23.6	1.15	1.01(88%)	0.94	0.83(88%)

[a]Turnover of one retention volume requires 11 h.
[b]Numbers in parentheses are percentage MPS recovered.

test organisms that are subjected to steep pollutant gradients. In 1977, an Aquatic Gradient Avoidance Response System (AGARS) was developed at ERL,GB to eliminate these limitations.

The system allows animals to choose between one uncontaminated zone and three increasingly toxic zones in a gradient trough that is monitored for extended time periods by infrared light sources, sensors, and a microprocessor (Fig. 12). Baseline data are obtained by comparing the time test animals spent in trough areas (avoidance or attraction) in the absence of a pollutant.

In initial tests with the system, a group of 4 pinfish (Lagodon rhomboides) were monitored in the trough for (1) 3 days in clean water; (2) 1 day in chlorination-

Table 14. Transformation of ^{14}C-methyl parathion in continuous-flow systems with sediment.

Hours	Unsterile System		Sterile System[a]	
	$^{14}CO_2$[b]	Polar Products[c]	$^{14}CO_2$	Polar Products
48	374	7.2	0	5.7
72	2720	15.3	63	8.6
96	3200	13.1	0	10.0
120	6952	22.1	96	11.4
144	6903	27.3	0	16.0
168	17693	30.7	0	16.0
192	23079	32.2	0	15.1
216	26314	31.8	0	15.8

[a]Treated with 2% formalin in reservoir and sediment.
[b]$^{14}CO_2$ (expressed as dpm) in alkaline traps per day.
[c]Percentage of total radioactivity in water column not extractable with methylene chloride.

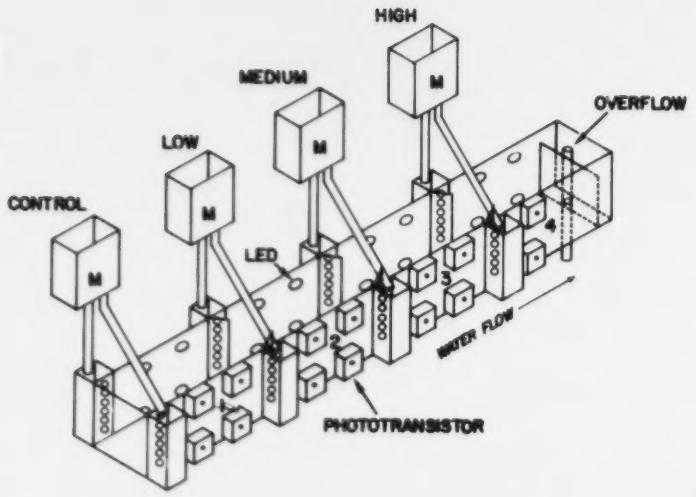

Figure 12. The Aquatic Gradient Avoidance Response System (AGARS), above, was developed to monitor behavior of marine animals exposed to toxicants.

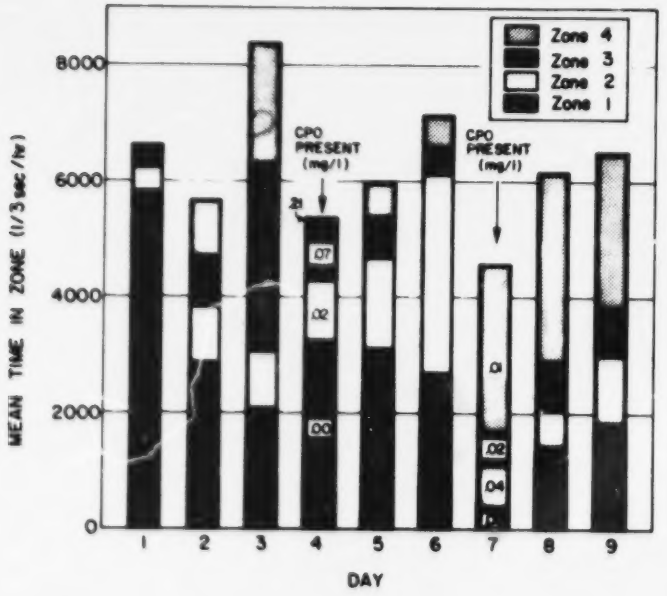

Figure 13. Results of 9-day AGARS test to monitor response of pinfish to toxicants. Mean hourly total of time spent in four zones are recorded. Chlorination-produced oxidants (CPO) were used on days 4 and 7; no toxicant was used during remaining test days.

produced oxidant (CPO) concentrations that progressively increased downstream (control, 0.02, 0.07, and 0.21 mg/ℓ for zones 1-4, respectively); (3) 2 days in clean water; (4) 1 day in CPO concentrations decreasing downstream (0.09, 0.04, 0.02, and 0.01 mg/ℓ in zones 1-4); and (5) 2 days in clean water. Trough temperature was between 28.5° and 29.0°C and salinity from 22 to 26 parts per thousand (ppt) for the 9-day test.

On day 1 fish spent the longest time in zone 1 where current was lowest; fish were more uniformly distributed throughout the four zones on days 2 and 3 (Fig. 13). No measurable CPO was present in the trough during this 3-day acclimation period.

On the day after CPO was added to the water (day 4), pinfish spent significantly ($\alpha \leq 0.05$) less time in zones 3 (0.07 mg/ℓ) and 4 (0.21 mg/ℓ) where CPO concentrations were highest. No avoidance of zone 2 (0.02 mg/ℓ) was evident. More residence time was recorded in zones 3 and 4. The avoidance threshold was indicated to be between 0.07 and 0.02 mg/ℓ. Return to CPO-free water on days 5 and 6 resulted in a continued high presence in zones 1 and 2.

On day 7, the second toxicant exposure day, zones 1 (0.09 mg/ℓ) and 2 (0.04 mg/ℓ) were significantly ($\alpha < 0.05$) ($\alpha < 0.05$) avoided; no avoidance was shown in zone 3 (0.02 mg/ℓ), indicating a fish avoidance threshold of approximately 0.02 to 0.04 mg/ℓ CPO. For days 8 and 9 (CPO-free), fish spent more time in zone 4 than in any other area.

Initial tests with AGARS showed that pinfish will avoid chlorination-produced oxidants at concentrations of 0.02 to 0.04 mg/ℓ. Future studies with AGARS will monitor the number to entries into zones by test species and time spent in each zone. Other possible uses for the device are tests for thermal or salinity preferences.

The system is currently being used at ERL,GB to monitor burrowing habits of pink shrimp exposed to toxicants. Earlier acute bioassays indicated that this species, which normally burrows during daylight and emerges at night, reduces time spent in burrows after exposure to certain poisonous compounds. This altered behavior could cause shrimp to become more vulnerable to predation. Preliminary tests have demonstrated the usefulness and versatility of AGARS in determining sublethal effects of toxicants on marine species.

Benthic Bioassay System

N.I. RUBINSTEIN, Research Biologist EPA Grant R804458, University of West Florida; C.N. D'ASARO, Principal Investigator; F.G. WILKES, Project Officer

Bioassays currently used by regulatory agencies and industry to assess the potential impact of pollutants on marine biota utilize a variety of sensitive pelagic and epibenthic species, but rarely incorporate infaunal organisms. This practice is due in part to the relative lack of sensitivity displayed by many infaunal species and the difficulties encountered in observing biological effects while organisms are buried in bottom sediment.

However, many infaunal species are deposit feeders that affect the marine environment by their substrate reworking activity. These organisms influence benthic community trophic structure and sediment stability and provide pathways for cycling nutrients, organic material, oxygen, and pollutants between the sediment and water column. Consequently, an accurate appraisal of the environmental effect of contaminants must include information regarding their impact on representative benthic species.

The Benthic Bioassay System was developed to examine the effect of low pesticide concentrations on the activity of an infaunal polychaete, Arenicola cristata Stimpson (commonly referred to as the lugworm). Lugworms are tube-dwelling deposit feeders found in littoral habitats throughout the world. Their activities are somewhat analogous to those of earth worms and are responsible for bioturbations of the substrate to depths as great as 40 cm. Populations of the European lugworm (Arenicola marina) have been observed to turn over nearly 500 tons of sediment (dry weight) per acre per year. Consequently, lugworms play an important role in sediment-water column dynamics and are of interest in evaluations of the impact of toxic substances on the benthic community.

Lugworms rework sediments as a function of their feeding mode, producing distinct topographical features in the form of funnel-shaped depressions on the substrate surface. Feeding is an integral part of an activity cycle believed to be controlled by internal pacemakers and therefore to be independent of normal environmental variables. A decrease in the production of feeding funnels indicates an interruption in the lugworm activity cycle.

The test developed at ERL,GB in 1977 monitors substrate reworking activity of lugworms by time-lapse photography. Rates of feeding funnel formation (surface area [cm2] turned under per hour) were determined with lugworms exposed to a toxicant (methyl parathion) and non-exposed (control) lugworms.

Experiments were conducted in aerated 125-ℓ glass tanks (0.25 m^2 surface area) containing 25 cm of clean beach sand (particle size 200 to 800 μm) and 75 ℓ of filtered seawater (20 μm filter). All tests were conducted under static conditions; salinity ranged from 22 to 25 ppt and temperature fluctuated between 23° and 25°C.

Six lugworms of similar size were introduced into each of two tanks and allowed 48 h to acclimate to test conditions. Seventy grams of ground seagrass added to each tank served as food and also provided contrast for photographs against the white sand in areas disturbed by feeding animals. The test compound (methyl parathion) was then introduced into one tank while the non-dosed tank served as the control. A 35-mm automatic camera was positioned above each tank and photographs of the substrate surface were taken at 12-h intervals for a 144-h

period. The photographs were then analyzed to determine the surface area (cm^2) disturbed by feeding animals. The total surface area turned under (disturbed) was plotted against time to give a substrate modification rate for exposed and control animals. Rates were subjected to linear regression analysis, and the slopes of the calculated lines were compared. Differences due to treatment were considered significant at $\infty = 0.01$.

After initial tests with methyl parathion, the procedure was used in 1977 to determine how three additional pesticides (sodium pentachlorophenol, Kepone, and Sevin) affect lugworm substrate reworking activity. Kepone was found to be most toxic to lugworms: mortality occurred after 144-h exposure to 29.5 µg/ℓ seawater; a significant effect on substrate reworking was observed at concentrations as low as 2.8 µg/ℓ seawater. Methyl parathion affected lugworm activity at concentrations between 75 and 150 µg/ℓ seawater (Fig. 14). Sodium pentachlorophenol inhibited lugworm activity at 45 to 80 µg/ℓ seawater. Sevin was found to be least toxic to lugworms. A reduction in substrate reworking activity was observed at 1,000 µg/ℓ seawater, although mortality did not occur until 10,000 µg/ℓ seawater.

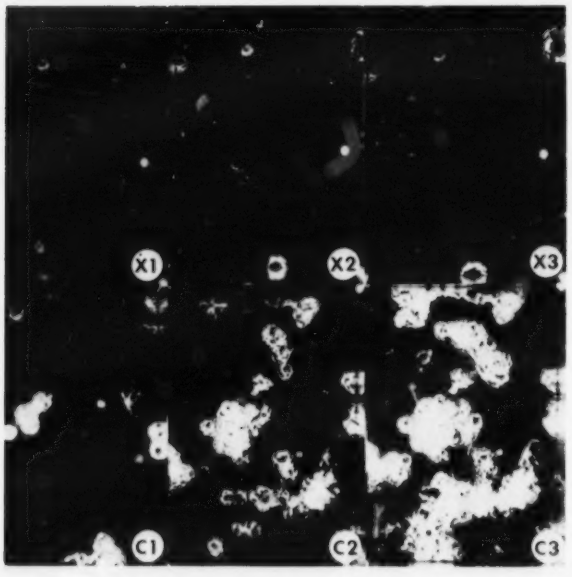

Figure 14. Lugworm feeding-funnels illustrated in above photographs were produced in (1) 24 h; (2) 72 h; (3) 144 h. X indicates action of lugworms exposed to 150 parts per billion methyl parathion; C indicates control lugworms.

Test results indicate that this technique can demonstrate a behavioral effect on infaunal organisms at sublethal concentrations of pollutants.

Estuarine Laboratory Systems

C.N. D'ASARO, Investigator. EPA Grant R804458, University of West Florida; F.G. WILKES, Project Officer

A microcosm, designed to simulate some trophic relationships in an estuarine grassflat community, was assembled to provide a vehicle in which fate and effects of xenobiotics could be tested. Selection of species for the microcosm (assembled in 39-ℓ aquaria) was based on these criteria: wide geographic distribution, abundance, simple culture methods and sensitivity to xenobiotics. Benthic diatoms, the estuarine grass Ruppia maritima, and detrital Ruppia formed the basis for the trophic structure. An isopod, Ericsonella attenuata, grazed on the grass and epiphytic microorganisms. An infaunal polychaete, Arenicola cristata, which is a detrivore, occupied the substrate. After acclimation, aquaria stocked with animals and operated at constant temperature conditions did not show evidence of environmental degradation in terms of the parameters measured (dissolved oxygen, pH, NH_3-N, algal cells/ml) for two weeks.

After behavior of the organisms in the system was determined, mortality or loss of specimens prior to termination of the experiments was prevented. In the final series of experiments, other invertebrates were successfully introduced into various tropic niches in the microcosm. A polychaete, Polydora ligni, was used to filter particulate material from the water. A herbivorous-detritivorous prosobranch, Bittium varium, scoured the grass blades and the aquarium walls. An apodid holothurian, Leptosynapta inhaerens, competed for detritus. Prior to termination of the experiment, juvenile portunids, Callinectes sapidus, were introduced as carnivores. The microcosm is now available for future tests with xenobiotics.

Predator/Prey System

J.A. FARR, Research Biologist. EPA Grant R804458, University of West Florida; C.N. D'ASARO, Principal Investigator; F.G. WILKES, Project Officer

Estuarine ecosystems contain vital species interactions, many of which may be vulnerable to stress by pollutants. A test was developed to measure the effect of a pollutant on an important estuarine predator/prey relationship. Grass shrimp, Palaemonetes pugio, were exposed to sublethal concentrations of methyl or ethyl parathion to determine if their ability to escape predation by the gulf killifish, Fundulus grandis, was impaired.

Table 15. Summary of the behavior of Fundulus grandis when preying on Palaemonetes pugio exposed to methyl and ethyl parathion as compared to undosed shrimp. Values differ from the control at the 0.05 ([a]) or 0.01 ([b]) level of significance, as determined by the Wilcoxon sign rank statistic*

	72-h exposure to 0.1 µg/ℓ ethyl parathion (N = 20)		24-h exposure to 0.5 µg/ℓ ethyl parathion (N = 19)		72-h exposure to 0.1 µg/ℓ methyl parathion (N = 18)		24-h exposure to 0.5 µg/ℓmethyl parathion (N = 19)	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Mean number of minutes taken to capture second and third shrimp	6.2	4.0[b]	6.5	4.2[b]	5.9	3.9[b]	7.5	4.5[b]
Mean number of shrimp consumed in fifteen minutes	3.7	4.3[a]	3.4	4.4[b]	3.3	4.1[b]	3.4	3.9[a]
Mean number of shrimp consumed in three hours	8.1	8.1	8.1	7.6	7.1	7.2	7.4	7.1

Estuarine ecosystems contain many vital species interactions, many of which may be vulnerable to stress by pollutants. A test was developed to measure the effect of a pollutant on an important estuarine predator/prey relationship. Grass shrimp, Palaemonetes pugio, were exposed to sublethal concentrations of methyl or ethyl parathion to determine if their ability to escape predation by the gulf killifish, Fundulus grandis, was impaired.

Two qualitative changes were noted in the behavior of the shrimp. Typically, in the presence of a predator, undosed shrimp remained motionless in a corner of the aquarium, but exposure to parathion altered this behavior. Rather than remaining immobile for long periods, the exposed shrimp continually moved around the tank and were thus more easily observed by the fish.

A second apparent effect was a decrease in the actual physical endurance of the shrimp. While actually fleeing a pursuing fish, exposed shrimp appeared to fatigue more quickly than unexposed shrimp. Their tail-snap escape reflex was less vigorous and continued for shorter time periods.

Shrimp exposed to low doses of parathion were caught more quickly than undosed shrimp (Table 15). More fish were consumed by the fish in the initial 15 minutes of the test, but no difference occurred in the total exposed and unexposed shrimp consumed throughout the experiment. These data indicate that exposure to sublethal doses of parathion renders shrimp more easily captured by killifish, but does not alter total shrimp consumption. However, grass shrimp, if more easily captured than other prey, would be expected to fulfill a greater proportion of a predator's diet.

Thus, predictable results are: (1) a reduction in detrital breakdown and in food chain efficiency at the lower trophic levels and (2) an increase in density of other prey species. Such changes in a multi-prey community could result in altered predator preferences and subsequently change community structure.

SUBSTITUTE CHEMICAL PROGRAM

Fate and Effects of Atrazine in Salt-marsh Ecosystems

DONALD E. DAVIS, Principal Investigator, EPA Grant R803835, Auburn University; F.G. WILKES, Project Officer

In the third year (1977) of this research, the investigator examined the accumulation, degradation, and effects of atrazine in salt-marsh ecosystems. Model ecosystems were developed to study effects of atrazine on the grass, Spartina alterniflora, fiddler crab, periwinkle snail, mussel, and detritus. Effects of atrazine stress on a salt marsh will be compared with effects observed in the model ecosystems.

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During 1977, the rate of conversion of dried S. alterniflora was determined in a microcosm. After a 30-day test, the average percentages of plants converted to detritus were: 78, 68, and 67 for grass doused with 0, 10^{-6}, and 10^{-4}M concentrations of atrazine, respectively. Results suggest that atrazine may inhibit detrital formation.

In a second experiment, S. alterniflora was grown for 2 days in a nutrient solution containing ^{14}C-ring-labeled atrazine, followed by 3 days in an atrazine-free solution. S. alterniflora was dried for 2 h at 105°C and placed in a microecosystem for 30 days. The partially decomposed material (detritus) was collected, dried, and extracted with 80% methanol. Percentage totals of ^{14}C in the extractions have yet to be determined.

In a third investigation, 3 groups of 15 crabs for 30 days fed on detritus derived from uncontaminated S. alterniflora. After drying, the detritus was wetted with 0, 10^{-6}, and 10^{-4} solutions of atrazine. Neither atrazine solution had any significant effect on crab mortality, behavior, or general appearance. In another 30-day period, 45 crabs fed on detritus from plants grown in ^{14}C-ring-labeled atrazine. Radioactivity of the feces increased steadily throughout the 30 days. Then 15 crabs were finely ground in a mortar by a pestle, and the ground material was extracted with 80% methanol. This solution was separated into chloroform and aqueous fractions and an insoluble residue that was hydrolyzed for 2 h with 0.5 N HCL. The hydrolyzed material was extracted with diethyl ether and then water. Percentages of radioactivity in crabs were: 4% in chloroform; 29% in first water extract; 2% in diethyl ether; 39% in second water extract; and 25% in residue. The remaining 30 crabs will be analyzed in the next reporting period.

In addition, research was initiated in 1977 on the effect of atrazine on the edaphic diatoms of the salt marsh. Mixed cultures of the diatom, Amphora sp., and a unicellular blue-green alga were isolated from soil samples from a Georgia salt marsh and exposed to 0, 10^{-8}, 10^{-7}, 10^{-6}, 5 x 10^{-6}, and 10^{-5}M for 2 h. Effects of these short-term treatments on subsequent photosynthesis (light fixation minus dark fixation) were measured: 91% inhibition of photosynthesis was caused by 10^{-5}M atrazine and 50% inhibition by approximately 5 x 10^{-6}M. Some tests showed a slight stimulation of photosynthesis at the two lowest atrazine concentrations. No effects were evident on dark fixation.

Thus far, the investigation has established that even fairly high levels of atrazine have little, if any, direct effect on S. alterniflora or crabs, or on the conversion of S. alterniflora to detritus.

Determination of the Environmental Impact of Several Substitute Chemicals in Agriculturally Affected Wetlands

J.W. DAY, Jr., S.P. MEYERS, and R.P. GAMBRELL, Principal Investigators. EPA Grant R804976, Louisiana State University; F.G. WILKES, Project Officer

The prime objective of this research is to find a means for solving land-use conflicts caused by chemical discharges from agriculture into wetlands. Investigators seek to determine: 1) fate of substitute chemical compounds (initially Guthion^{R}) in brackish wetlands, correlating results with past productivity, nutrient flows, application rates, and runoff patterns; 2) fate and effects of substitute pesticides applied to test plots; 3) persistence of Guthion under varying conditions. One year of a projected three-year study has been completed.

Investigators will examine the persistence of Guthion in swamp soil, using controlled soil physiocochemical parameters (pH, oxidation-reduction conditions, salinity).

In preliminary field studies, selected microbial populations were identified: aerobic heterotrophs, chitinoclastic bacteria, cellulolytic forms, proteolytic bacteria, amylolytic forms, NO_3 reducers, and yeast fungi. Studies were initiated on the effect of Guthion on 1) the growth response curve for selected heterotrophs, 2) chitin degradation, and 3) chitin solubilization.

Understanding the effects of pesticides in wetlands requires knowledge of the influence of hydrology, chemistry, temperature, and community structure. As important members of the food web, invertebrates are useful tools for detecting environmental perturbations. Some species, such as the crayfish (Procambarus clarki), are consumed by man. Invertebrates occupy virtually all trophic levels and as recycling agents can reflect environmental conditions for extended time. Therefore, field studies in this project focused on the effects of Guthion on invertebrate populations. The nature of the changes within these communities will need further study before conclusions can be drawn.

In addition, investigators initiated an attempt to measure the metabolism of communities occupying the swamp's floor (the floating vegetation layer, the water column, and sediments). In the forthcoming reporting period, controlled field applications of Guthion will be undertaken in isolated wetland areas. Studies will be conducted on the degradation of Guthion and other substitute chemicals (malathion and methyl parathion) in a salt marsh. Pesticide levels will continue to be measured in sediment, water, and biota.

MICROBIOLOGY

Aquatic microorganisms play a critical role in the metabolic transformation of organic and inorganic compounds entering lakes, estuaries, and oceans. Many of these compounds, particularly the chlorinated hydrocarbon group,

originate from man's industrial and agricultural enterprises. Often, they have no close counterpart in nature; therefore, their fate and accumulation are important in determining environmental quality.

Kepone Toxicity to Aquatic Microorganisms

A.W. BOURQUIN, Research Microbiologist;
P.H. PRITCHARD, Environmental Scientist

Chlorinated hydrocarbons and pesticides are known to accumulate in humus soil and aquatic ecosystems, due in part to their resistance to biological and non-biological degradation.

Questions remain unanswered regarding the potential of these compounds to alter basic microbial transformation processes. Therefore, the impact of Kepone on microbial transformation processes in the James River, Virginia, was investigated. This study was conducted in four phases: 1) pure culture toxicity; 2) natural population toxicity; 3) effects on oxygen uptake; 4) effects on naphthalene and methyl parathion transformation.

In the first phase, toxicity of Kepone to laboratory stock cultures was determined by the disc agar diffusion sensitivity method. The stock cultures were originally isolated from batch culture by selective enrichments on a wide spectrum of substrates. Of the 30 isolates tested, 33% were inhibited at the 3.65 µg/disc concentration, and 47% were inhibited at the 14.6 µg/disc concentration.

Kepone-sensitive cultures showed no significant difference from randomly selected stock cultures in terms of morphology, aliphatic hydrocarbon utilization, tolerance to pesticides (Aroclor 1242 and 1016 methoxychlor, heptachlor, DDT, malathion, toxaphene), lipolytic and proteolytic activity, nitrate reduction, sugar utilization, and urea hydrolysis. However, many of the sensitive cultures (7/8) were gram positive, whereas fewer of the Kepone-tolerant cultures (2/9) were gram positive.

The same was true of amylolytic activity: 6/8 were positive for Kepone-sensitive cultures and 1/7 were positive for Kepone-tolerant cultures. A greater percentage (50% versus 11%) of Kepone-sensitive bacteria metabolized one or more aromatic compounds (phenol, naphthalene, toluene, biphenyl, and xylene). Only one of the six fungal cultures was poisoned by Kepone at the 14.6 µg/disc concentration. Two yeasts, Candida maltosa and lipolytica, were both sensitive, but at a higher concentration than the bacteria tested.

Toxicity of Kepone to natural mixed populations of bacteria from a variety of marine habitats was determined by standard total viable counts and Zobell's seawater agar containing dissolved Kepone. Kepone concentrations (as low as 20 µg/ℓ) inhibited development of colonies on the agar plate. Different degrees of inhibition were noted in samples taken from the same area at different times; frequently, concentrations below 20 µg/ℓ appeared inhibitory. These levels of Kepone toxicity almost equaled those found in James River sediment.

A total of 17 colony-forming units, which grew in the presence of Kepone, were selected on the basis of predominance for further study. When compared with 20 isolates randomly selected from Zobell's marine agar (no Kepone), 75% of the Kepone-tolerant isolates displayed amylolytic and lipolytic activity, whereas only 55% of the non-tolerant isolates exhibited such activity; 90% of the Kepone-tolerant isolates were gram negative, compared to 55% of the isolates from Zobell's marine agar (no Kepone).

The toxicity of Kepone to bacteria isolated from sediment was studied by using standard viable plate counts on Zobell's marine agar containing Kepone; colony-forming units incubated under anaerobic conditions showed no significant change in the number relative to aerobic controls, thus implying a resistance to Kepone under anaerobic conditions.

The inhibition of oxygen uptake appeared to be a sensitive indicator of Kepone toxicity. Isolates showed varying degrees of sensitivity: most were inhibited at the 20 mg/ℓ concentration, whereas relatively few were inhibited at the 2 mg/ℓ concentration. Additionally, studies showed that Kepone in some cases actually stimulated oxygen uptake.

Eco-core experiments have shown that both the rate and extent of degradation of naphthlene and methyl parathion in the presence of Kepone were reduced relative to non-Kepone containing controls.

Overall, these experiments indicate that Kepone is toxic to microorganisms. To date no chemical or biological degradation of Kepone has been demonstrated, and Kepone appears to be only very slowly washing out of the James River system. Residual Kepone possibly could inhibit bacteria and the processes they mediate.

Surface Microlayer Studies

A.W. BOURQUIN, Research Microbiologist

Surface microlayer studies are concerned with the microbial ecology and the chemistry of organic material that accumulates on water surfaces. Past investigations at ERL,GB have shown these surface films to be active biologically and rich in organic pollutants, i.e., chlorinated aromatic hydrocarbons and polychlorinated biphenyls (PCBs). Experiments in 1977 focused on: (1) the movement of organic compounds from the water column into the surface film and (2) artificial laboratory surface microlayers.

In the first phase, special vessels were constructed to sample both a hexadecane surface film by the Nuclepore^{R} membrane technique and the water beneath the suface film. Movement of the compound into the surface film was studied by spiking the water with radioactive compounds. Tests with methyl parathion and DDT showed that pollutants rapidly concentrate in surface microlayers: in less than 48 h, methyl parathion concentrated in the surface film 150 to 200 times the amount observed in the water column (on a weight for weight basis); DDT concentrated from 250 to 300 times the amount found in the water column for this time period. Concentrations

rapidly reached equilibrium; however, the amount accumulated was directly proportional to the partitioning coefficient of the compound between seawater and the hexadecane film.

In the second phase of the research, tests using hexadecane as a basic surface film showed that the addition of alkyl benzene sulfonate, tannic acid, or Tween-80 all increased the concentration of DDT from the water column, i.e., 260 to 320 times that seen in the water column.

Effects of Pollutants on Microbial Activities in Estuarine Surface Films

DONALD AHEARN, Principal Investigator. EPA Grant R804477, Georgia State University; A.W. BOURQUIN, Project Officer

A group of compounds that earlier had reacted positively in mutagenicity tests (Ames systems) were used in studies of 9 surface slick isolates. In tests using the disc diffusion method, pentachlorophenol (PCP) and Captan inhibited the growth of 9 and 8 of the 9 isolates, respectively. Further tests with discs saturated with 0.1 ml of DMSO containing 100 µg of test substance showed that PCP inhibited only 3 of 9 isolates, whereas Captan and Captafol inhibited all 9 cultures. Future studies will establish the dose responses for selected inhibitory compounds on Ames strains and on surface slick isolates.

Previous studies suggest that heptachlor may alter the transport of hydrophobic materials into the cell. Therefore, studies undertaken in 1977 assessed the synergistic action of heptachlor and mutagenic compounds in the Ames system. Subinhibitory levels of chlordane (20 ppm) were found to be inhibitory in the presence of heptachlor (20 ppm). No enhanced mutagenicity was noted for combinations of heptachlor with BHC, Captafol, Captan, Carbaryl, chlordane, Kepone, mirex, and PCP. Preliminary results from surface film samples indicate the presence of toxic and possibly mutagenic substances.

Earlier, this investigation demonstrated preferential removal of naphthalene and biphenyl in a synthetic oil, using a heptachlor (1 mg/ml) system inoculated with either Candida lipolytica or C. maltosa. Work in 1977 examined the disappearance of naphthalene from a simplified system containing only hexadecane or hexadecane and biphenyl. Biphenyl appeared to stimulate utilization of hexadecane and naphthalene by C. lipolytica. Heptachlor reduced naphthalene utilization by both yeasts. Similar results were observed for a modified synthetic oil containing biphenyl, naphthalene, tetradecane, hexadecane, and pristane. Heptachlor (1000 ppm) or Kepone (10 ppm), when added into this system, caused a reduction in utilization of naphthalene and hexadecane.

Tests on effects of sublethal concentrations of pesticides on proteolytic activity showed that selected aromatic and chlorinated hydrocarbons caused no reduction in this enzyme activity.

Seventeen cultures were tested for their response to combinations of PCP, o-chlorophenol, naphthalene, 1-chloronaphthalene, heptachlor, and methoxychlor. Synergistic responses were often produced by combinations of 1-chloronaphthalene and heptachlor or methoxychlor (8 out of 10); napthalene and PCP and naphthalene and ortho-chlorophenol also inhibited these cultures.

Biodegradation of Chlorinated Dibenzodiozins and Dibenzofurans

D.T. GIBSON, Principal Investigator. EPA Grant R804525, University of Texas; A.W. BOURQUIN, Project Officer

Chlorinated dibenzo-p-dioxins have long been recognized as possible by-products in the manufacture of certain chlorinated phenols (i.e. pentachlorophenol and 2,4,5-trichlorophenol). Interest in these compounds has increased since the discovery of highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in samples of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Further, chlorinated dibenzo-p-dioxins and dibenzofurans have been detected in commercial preparations of polychlorinated biphenyls. Many of these chlorinated compounds are used extensively in both industry and agriculture; therefore, the high level of toxicity of the chlorinated dibenzo-p-dioxins is an environmental concern.

This project seeks to determine reaction microorganisms that degrade dibenzofuran and dibenzodioxin, and to identify their chlorinated derivatives.

Microorganisms were collected from mud flats and algal mats at Port Aransas, Texas. Each sample was incubated in seawater containing either dibenzo-p-dioxin or dibenzofuran (0.5 g/ℓ). No growth was obtained after 10 weeks incubation at 25°C on a rotary shaker (250 rev/min). Six additional estuarine samples (collected later) also failed to yield any organisms that could grow by using either substrate as the sole source of carbon and energy. The same samples, however, yielded growing organisms that were able to use hexadecane as their sole source of carbon. Nine of these organisms were retained for co-oxidation studies.

Other laboratory cultures, however, were found to metabolize dibenzo-p-dioxin and dibenzofuran: a Beijerinckia species previously isolated through its ability to grow in biphenyl; a Pseudomonas species that degrades naphthalene; and Cunninghamella elegans, an organism isolated through ability to degrade crude oil from North Carolina estuary. These microorganisms would not grow in dibenzo-p-dioxin or dibenzofuran. However, when an alternative growth substrate was present, significant dedegradation of both aromatic compounds occurred.

Although no organism was found to use dibenzo-p-dioxin and dibenzofuran as a sole source of carbon and energy, these compounds were readily metabolized when an alternative carbon source was available.

Dibenzo-p-dioxin was oxidized to cis-1,2-dihydroxy-1,2-dihydrodibenzo-dioxin, which then forms a catechol. Studies have yet to determine whether the molecule can be degraded completely to naturally occurring products.

Dibenzofuran is attacked at two positions on the mol-

ecule, indicating that dihydrodiols are produced at the 1,2- and 3,4-positions. Identification of the degradation products posed significant analytical problems, although most have been solved. Preliminary evidence indicates that two catechols and two ring-fission products are produced by Beijerinckia. The broad specificity of this organism (in terms of its ability to metabolize the same molecule at different positions) heightens interest in forthcoming studies on the effects of chlorine substitution.

The fungus Cunninghamella elegans appears to metabolize aromatic compounds in a manner analogous to the mammalian liver. Mammals are known to produce arene oxides which, in some cases, can react with nucleic acids to initiate mutagenic changes. In the case of benzo[a] pyrene, a metabolite, trans-7,8-dihydroxy-7,8-dihydrobenzo[a] pyrene, serves as a substrate for the formation of 9,10-epoxy-7,8-dihydroxy-7,8-dihydrobenzo[a] pyrene (diol-epoxide), which is both mutagenic and carcinogenic. Although no studies have been reported on the mammalian metabolism of dibenzofuran, results obtained in this study indicate the formation of an arene oxide. These molecules, by rearrangement, are known to given phenols and undergo enzymatic hydration to form trans-dihydrodiols. Because C. elegans produces 2- and 3-hydroxydibenzofuran and 2,3-dihydroxy-2,3-dihydrodibenzofuran, the prior formation of 2,3-epoxydibenzofuran is indicated. Also, the stability of the dihydrodiol to acid treatment indicates that it is the trans-isomer.

Metabolites isolated in sufficient amounts will be tested for mutagenic activity in the Ames system by Dr. Eugene Goldschmidt at the University of Houston during the next reporting period.

Insecticide Persistence in Natural Seawater as Affected by Salinity, Temperature, and Sterility

W.W. WALKER, Principal Investigator. EPA Grant R803842, Gulf Coast Research Laboratory, Ocean Springs, MS; A.W. BOURQUIN, Project Officer

This investigation determined the effect of temperature, salinity, and sterility on the persistence and degradation of representative organophosphorus and chlorinated hydrocarbon insecticides (malathion, parathion, methyl parathion, diazinon, and methoxychlor).

Surface water samples of 0, 10, 20, and 28 parts per thousand (ppt) salinity were amended with the above insecticides and incubated in the dark at 30°, 20°, and 10°C under sterile and nonsterile conditions. Insecticide abatement was followed by electron-capture, gas-liquid chromatography.

No significant differences between sterile and nonsterile treatments were observed for any insecticide. The effect of increasing temperature was highly significant with regard to increased degradation of malathion, parathion, methyl parathion, and diazinon. Methoxychlor reflected the recalcitrance characteristic of the chlorinated hydrocarbon insecticides throughout 84 days of incubation and was not significantly affected by salinity, temperature, or sterility. Salinity effects varied among the four organophosphates: highly significant for malathion and diazinon, significant for methyl parathion, but not significant for parathion.

Malathion was the shortest-lived insecticide: half-lives at 30°C varied from approximately 11 days in fresh water to less than two days at 10, 20, or 28 ppt salinity. The disappearance rate of methyl parathion was second only to malathion and ranged from 27 days (half-life) in fresh water to 16 days at 28 ppt. In fresh water, a 45-day half-life for diazinon suggested a substantial resistance to degradation, especially at 30°C. In saline water, however, diazinon abatement was accelerated; 24 days half-life at 28 ppt salinity. Parathion, the most persistent organophosphate insecticide, reflected a half-life of at least 44 days regardless of salinity.

One bacterium, tentatively identified as Moraxella sp., was isolated from sediment by enrichment and proved capable of readily degrading malathion either as a primary carbon source or in the presence of peptone. Two bacteria were tested for the ability to degrade methyl parathion: one bacteria, possibly a Pseudomonas sp., proved capable of utilizing the insecticide with or without peptone; the other, a Moraxella, reflected no degradation of methyl parathion as the primary carbon source and only limited utilization of the insecticide in the presence of peptone. Neither bacteria screened for parathion metabolism was capable of insecticide degradation under conditions of this evaluation.

Work was completed September 30, 1977, on this two-year study. The final will be published early in 1978 in the EPA Ecological Research Series.

Feasibility of Using Bacterial Strains to Test for Environmental Carcinogens

J.E. EVANS, Principal Investigator, EPA grant R804586, University of Houston, Houston, TX; A.W. BOURQUIN, Project Officer

A rapidly growing amount of data is available concerning the mutagenic and carcinogenic properties of new chemicals and products manufactured for commerce in recent years. However, literature pertaining to mixtures, such as chemical wastes, is scarce and difficult to locate.

This grant produced a review of literature related to the feasibility of using bacteria as screening agents to detect cancer-causing agents in the environment. Mutagenicity data were also included in the literature search because of growing experimental evidence that most chemical carcinogens are mutagens and therefore many mutagens may be carcinogens.

Results of the investigation indicate that bacterial strains can be used to initiate a series of studies aimed at screening mixed wastes for potential mutagens and carcinogens. Findings will be published in the EPA Ecological Research Series in 1978.

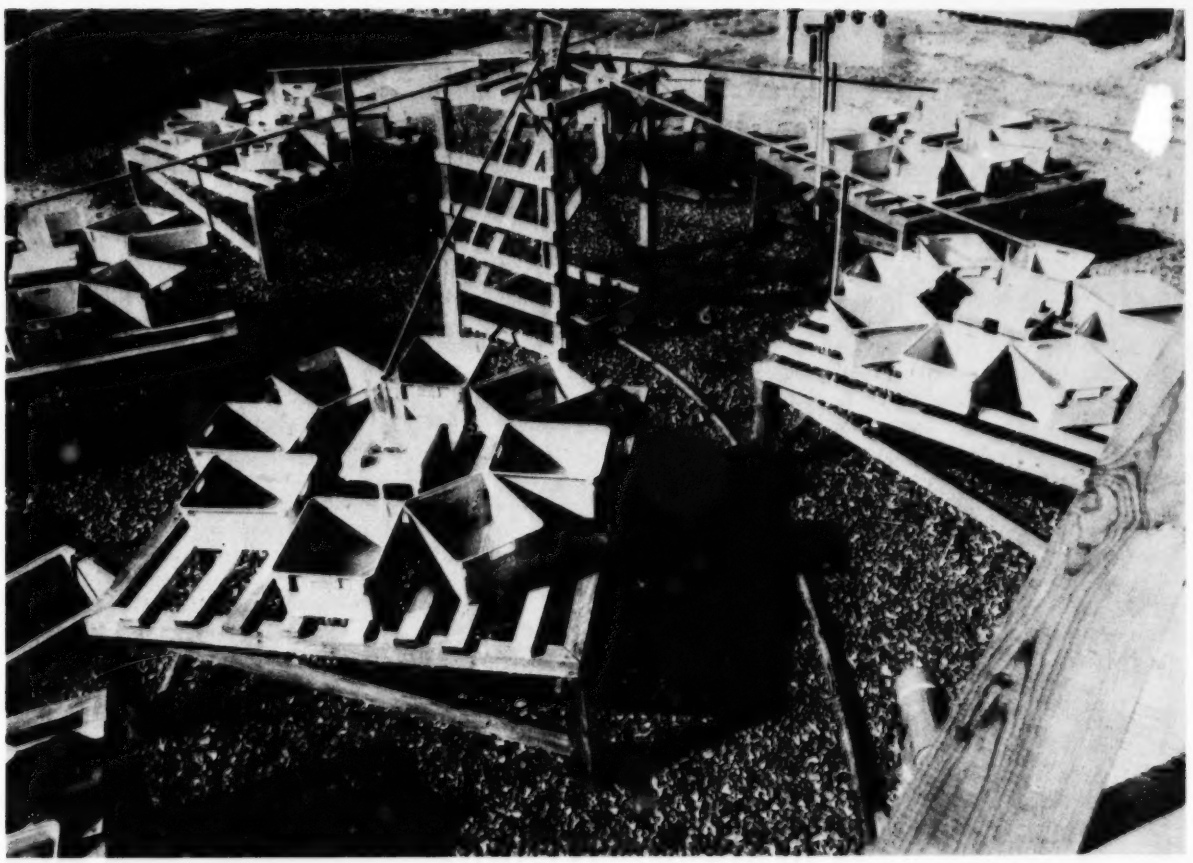


Figure 15. Marine Ecosystem Test Units (METU) at the Bears Bluff Field Station enable scientists to observe effects of chlorination on marine communities indigenous to adjacent South Carolina salt marshes.

BEARS BLUFF FIELD STATION

WILLIAM P. DAVIS, Chief

The Bears Bluff Field Station, located 25 miles southwest of Charleston, South Carolina, on the relatively pristine North Edisto estuary, conducts and coordinates research related to the impact of biocides and disinfections on marine/estuarine organisms, experimental communities, and food webs.

Since designated a branch of ERL,GB in 1974, the field station has been engaged in research related to the effects of biocide oxidants and by-products resulting from their application in the marine environment. In 1977, the staff investigated new methods for chemical analyses adaptable to testing the natural marine/estuarine waters and related aquatic communities for toxicity, histopathology, and behavioral responses. These studies are designed to yield better assessment of the transfer and transformation of compounds entering the marine food web.

Analytical Chemistry, Biochemistry, and Productivity Studies

A.M. CRANE, Chemist;
S.J. ERICKSON, Research Aquatic Biologist;
S. KLINGENSMITH, Biologist

Chemical analyses at the Bears Bluff Field Station in 1977 were conducted primarily on by-products produced by direct chlorination and interactions with natural levels of ammonia and resulting halogenated amines.

Results of a one-year study completed in 1977 showed that continuous chlorination of flowing seawater containing marine plankton caused a measurable reduction in the concentration of adenosine triphosphate (ATP), sometimes used as an indicator of living biomass.

Effects of continuous chlorination on planktonic life were measured by ATP content in two flowing seawater systems for one year. System A consisted of 96 37- ℓ aquaria; System B, 40 5.5-ℓ aquaria.

The mean control ATP for System A was 0.55 µg/ℓ; the measured ATP was reduced to 87.1% of mean control value in aquaria treated with 0.125 mg/ℓ. (At this nominal level, no measurable residual is present.) Concentrations of 0.125 mg/ℓ sodium hypochlorite reduced ATP levels to 77.6% of control, and 0.5 mg/ℓ sodium hypochlorite caused a 66.8% reduction in measured ATP levels.

In System B, mean control ATP was 0.40 µg/ℓ. Treatment with sodium hypochlorite resulted in the following percentage reductions of measured ATP levels: 0.47 mg/ℓ, 74.5%; 0.94 mg/ℓ, 56.7%; and 1.14 mg/ℓ, 42.5%.

Table 16. Fundulus heteroclitus: Locations of significant F-ratios for all stages of development and corresponding P values

Develop. Stages	Variables							
	A	B	C	AB	AC	BC	ABC	R
1-2 Cell	≤ .0001	NS	NS	NS	NS	NS	NS	NS
Gastrula	≤ .01	NS	NS	NS	NS	NS	.01	NS
Circulation	≤ .001	NS	NS	NS	NS	NS	NS	NS
10-day Embryo	≤ .0001	NS	NS	NS	NS	NS	NS	NS
0-day Larvae	≤ .001	≤ .05	≤ .001	≤ .01	≤ .001	≤ .001	≤ .001	NS
7-day Larvae	≤ .0001	≤ .0001	≤ .0001	≤ .05	≤ .0001	≤ .01	≤ .01	NS

A = Temperature
B = Duration of exposure
C = TRC
AB, AC, BC, ABC = Interactions
R = Replicates
NS = Not significant

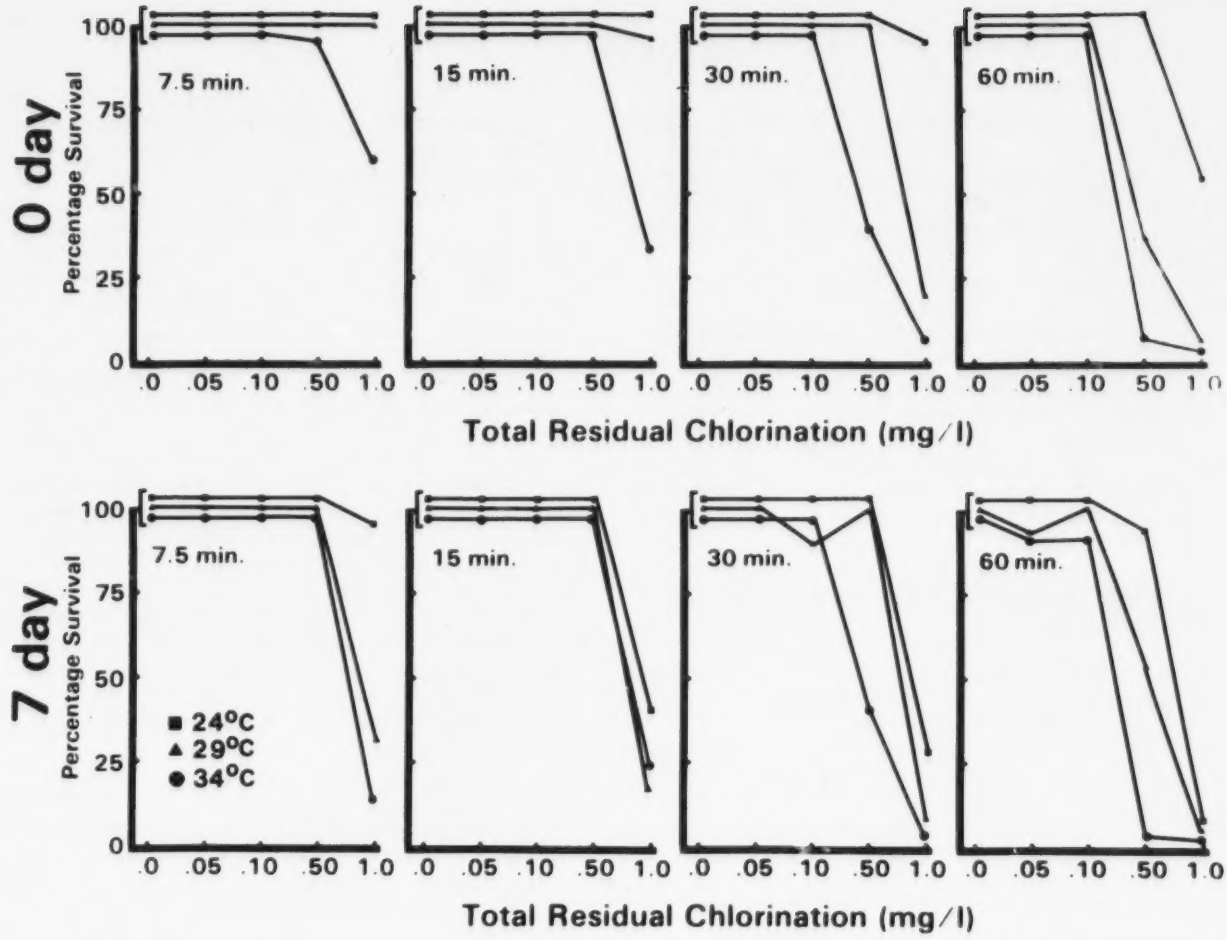


Figure 16. Percentage survival of 0-day-old and 7-day-old mummichog larvae subjected to different TRC concentrations at 3 temperatures for 4 time intervals

In another study conducted in 1977, the Bears Bluff Field Station reported that certain commercial resin ion exchange filters used in laboratories and analytical work in South Carolina had been contaminated by cleaning water. The investigation demonstrated the need to properly clean resins during treatment of water for laboratory use. These findings were reported in the EPA Ecological Research Series, "Water Softening and Conditioning Equipment," (EPA-600/3-77-107), September 1977.

Single Species Bioassays (Fishes)

D.P. MIDDAUGH, Research Aquatic Biologist

Research conducted in 1977 determined the sensitivity of embryos and young larvae of the mummichog (Fundulus heteroclitus) to different combinations of chlorination levels and thermal stress. Results indicate that 2 larval stages tested (0-day old and 7-day old larvae) were more sensitive than the 4 embryonic stages (Table 16).

The response of the 2 larval stages to 4 concentrations of oxidants produced by chlorination at 3 temperatures (24°, 29°, and 34°C) for 4 time intervals (7.5, 15, 30, and 60 min) are shown in Fig. 16. In general, larvae demonstrated a trend of increased sensitivity to total residual chlorination with increased exposure temperature and exposure time.

Toxicological responses observed in tests exposing freshwater and estuarine fish to chlorination levels appear

to be similar. However, little information is available on the physiological responses of marine fish exposed to chlorinated secondary sewage treatment wastes or chlorination applied as antifouling biocide in thermal electric generating plants. An investigation was undertaken at Bears Bluff to measure toxicological and other physiological responses of juvenile spot (Leiostomus xanthurus) exposed to various levels of chlorination.

Results showed that measured concentrations (0.09 to 0.37 mg/ℓ) of oxidants produced by chlorination caused rapid mortality of spot; the median lethal time (LT50) ranged from 88 to 400 min. Slightly lower oxidant concentrations (0.09 and 0.13 mg/ℓ) caused no deaths during 48-h exposure in flowing seawater (Table 17).

The pathobiology unit at ERL,GB studied the effects of chlorine on the gill tissues of exposed spot. When fish were exposed to 1.0 mg/ℓ$^{-1}$ NaOC1, sloughing of respiratory gill epithelium began (Fig. 17: 3,4,5). When exposed to 1.6 to 3.2 mg/ℓ chlorine, gill epithelium sloughed and fused; some gills underwent aneurysm development (became enlarged) (Fig. 17: 6,7,8). These findings suggest that the mechanism for death caused by chlorine in spot may be related to destruction of gill tissues by direct contact of chlorine on exposed respiratory surfaces.

Oyster Studies

G. SCOTT, Biologist;
D.P. MIDDAUGH, Research Aquatic Biologist

The oyster (Crassostrea virginica G.) was exposed to chlorination-produced oxidants (CPO) in different seasons (fall, winter, and spring) for 45 to 75 days in an investigation of seasonal survival patterns in 1977.

Survival was lowest in the higher nominal exposure concentrations (5.60 and 3.20 mg/ℓ NaOC1); at lower concentrations (1.80 and 1.00 mg/ℓ), survival often approached rates observed in the controls (93-100%). Toxicity of CPO to oysters appeared to be related to the seasonal water temperature and seasonal physiological condition of the oyster.

Exposure to CPO also resulted in an avoidance response: fecal production was significantly reduced in exposed oysters, suggesting oysters utilize alternate metabolic pathways to avoid direct exposure to chlorination.

Measurements of condition index (dry meat weight/cavity volume x 100) indicated that with increased periods of exposure to CPO, tissue production is significantly decreased. Gonadal index (dry gonad weight/dry meat

Table 17. Acute toxicity data for replicate tests with spot exposed to 5 nominal NaOC1 concentrations.

Replicate Test	Nominal NaOC1 Conc. mg l^{-1}	Measured CPO Conc. mg l^{-1} N	$\bar{x}$	s	LT50 (min)	95% C.I. (min)	Slope of Curve	95% C.I. of Curve
A	1.0	5	0.09	0.005	>2880	–	–	–
B	1.0	5	0.09	0.007	>2880	–	–	–
A	1.4	5	0.12	0.01	>2880	–	–	–
B	1.4	5	0.13	0.01	>2880	–	–	–
A	1.6	5	0.13	0.02	360	285-454	1.66	1.38-1.99
B	1.6	5	0.13	0.01	400	296-540	1.93	1.54-2.41
A	1.8	5	0.19	0.02	216	183-254	1.44	1.26-1.64
B	1.8	5	0.20	0.02	250	217-288	1.38	1.25-1.52
A	3.2	5	0.37	0.03	88	81-95	1.20	1.11-1.27
B	3.2	5	0.36	0.02	95	90-100	1.12	1.08-1.16

Control mortality <5% for all tests.

Measured mean ($\bar{x}$) CPO values and standard deviation(s) are given for each test.

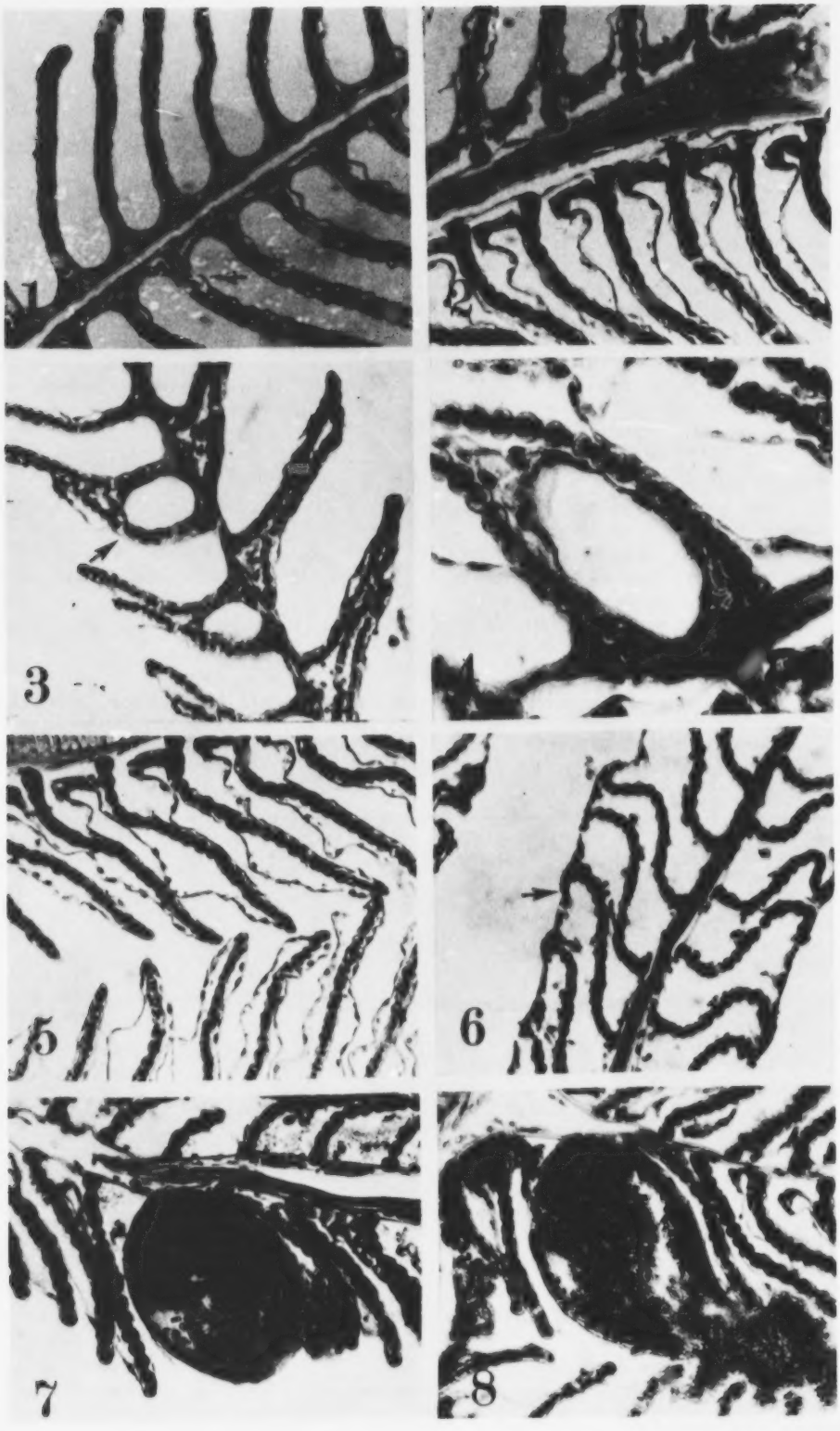
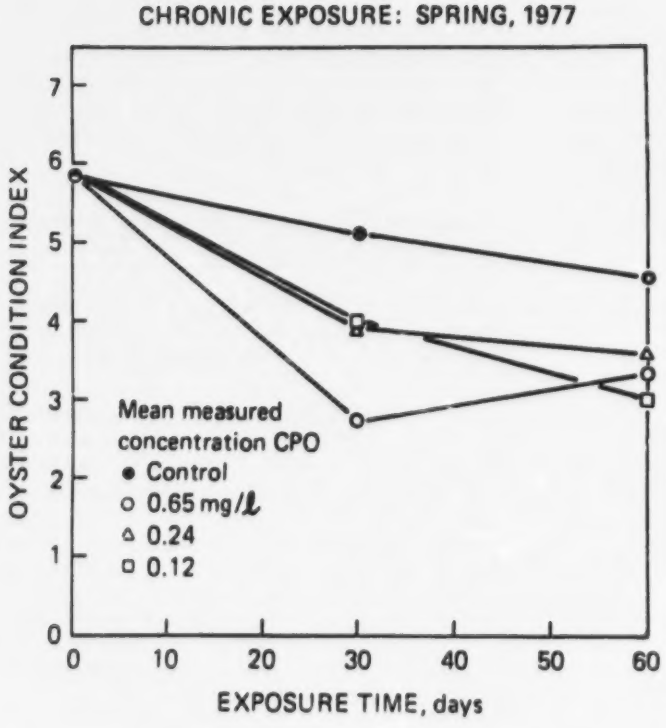
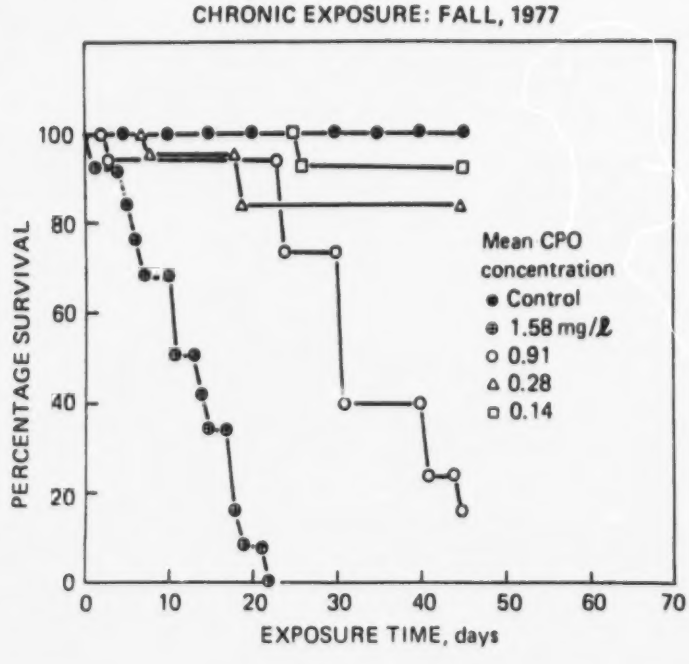


Figure 17. Spot gills became enlarged and tissues show progressive damage after exposure to CPO





Figures 18 and 19. Results of chronic exposures of oysters to CPO in spring 1977 (60 days) and fall 1977 (45 days). Ambient temperatures were maintained throughout tests.

trol tanks (without chlorination). Colonization of benthic taxa in the METU system apparently reflects the neutralization interaction between oxidants and hydrogen sulfide from organic complexes produced in test unit sediments (Fig. 20). In general, community density in high concentrations paralleled that in control. Results apparently reflected the threshold range for chlorination effects on these communities and on natural water.

METU will be modified in 1978 for studies of long-term effects and bioaccumulation of by-products of chlorination.

Mini-METU

ALSTON C. BADGER, Research Aquatic Biologist; I.B. JOHNSON, Biologist

In parallel experiments, a minaturized version of the testing system was evaluated. Analyses of results generally corroborated METU results. However, the Mini-METU system was less sensitive to the effects of chlorination because fewer organisms colonized in the smaller indoor units.

Halo-organic Compounds in Estuarine Waters

R. HELZ, Principal Investigator. EPA Grant R803839-01/02, University of Maryland; W.P. DAVIS, Project Officer

Halo-organic compounds resulting from chlorination were investigated under laboratory and field conditions. Haloforms were generated in the laboratory with chlorine doses of 1-10 mg/ℓ, the range employed by many coastal power plants.

This study, completed in 1977, showed that bromoform is the principal compound found in halo-organic by-products at salinities above 1 g/kg. On a molar basis, more than 4% of the chlorine from chlorination in these tests was converted to haloforms. In the laboratory, ozone also generated haloforms in estuarine water at rates similar to those obtained from chlorine. However, at a power plant field site, only traces of haloforms were found, apparently due to haloform-bypassing reactions or analytical techniques. The investigator did not identify any bypassing reaction, but suggested the formation of stable halogenated macromolecules.

Isolation and Study of Halo-organics Resulting from Chlorination of Seawater

J.H. CARPENTER, Principal Investigator. EPA Grant R803893, Rosenstiel School of Marine and Atmospheric Science, Miami, FL; W.P. DAVIS, Project Officer

This investigation defined and corrected the anomaly in marine measurements with the aid of an amperometric titrator. Investigators also identified the photolytic production of bromate from seawater chlorination and verified the kinetics and rate of bromoform production from chlorination. Future work will emphasize food-web transfer of by-products of chlorination and analysis of metabolic transformation of compounds in organisms. A long inventory of halo-organics resulting from seawater chlorination is in preparation.

Design of Experiments, Statistical Analyses, and Evaluation of Aquatic Research Data

R.G. DOMEY, Principal Investigator. EPA Grant R805007, University of Texas, Medical School, San Antonio, TX; W.P. DAVIS, Project Officer

Data sampling, design consultation, and statistical analyses are provided under this grant. From the summer 1975 through fall 1977, METU weather, temperature, and chemical data were collected and processed for computer programming.

Data related to the reproduction, spawning, and hatching patterns of the Atlantic silversides were processed in collaboration with D. Middaugh. The information will be used in field tests in 1978 to validate predictions.

Statistical analyses were also conducted for studies on response of oysters to low-level chlorination and by-products of chlorination.

An Investigation of the Ecological Effects of Residual Ozone to Selected Estuarine Species

D.T. BURTON, Principal Investigator. EPA Grant R804683, The Academy of Natural Sciences of Philadelphia, Benedict Estuarine Research Laboratory, Benedict, MD; W.P. DAVIS, Project Officer

Research in 1977 indicated that ozonation of marine waters produced the same progression of bromination for organics as for chlorine. Calculation to adjust ozonation to levels equivalent with chlorination levels and rates were developed and applied at the outset; tests using resultant "chlorination-equivalents" revealed no difference in relative toxicity of exposed invertebrates or fish. Studies of physiological effects of ozone-induced halo-organics on selected species will continue in 1978.

Fish Webs, Populations, and Productivity in a Southeast Coastal Marine Marsh

N.W. CHAMBERLIN, Principal Investigator. EPA Grant R8044688, Grice Marine Biological Laboratory, College of Charleston, Charleston, SC; W.P. DAVIS, Project Officer

Selected study sites in the marshes of the Leadenwah/North Edisto River estuary were monitored in 1977 to evaluate marine communities and food webs. These waters

weight x 100) measurements demonstrated that the gonads are the site of tissue reduction and that gonadal glycogen reserves are depleted in oysters exposed to CPO.

Mantle tissue respiration measurements showed no significant differences in O_2 uptake between control and oysters exposed 30 days at 5°C. However, at 28°C, the O_2 uptake rate in oysters was significantly higher than in controls, suggesting a possible synergistic response between CPO and elevated water temperatures and a possible explanation for seasonal survival patterns (Figs. 18 and 19).

Marine Ecosystem Test Units (METU)

W.P. DAVIS, Supervisory Research Aquatic Biologist;
R.L. YOAKUM, Biology Technician;
H.R. FOULK, Biology Technician;
S.J. ERICKSON, Research Aquatic Biologist

Since 1975, Bears Bluff investigators have used the Marine Ecosystem Test Units (METU) (Fig. 15, p.32) to observe the complex interactions between low levels of chlorination and community structure of benthic taxa.

METU consists of 96 outdoor tanks that receive a continual flow of estuarine water containing entrained larval organisms. The system serves as a habitat for developing communities including algae (6 to 12 species), amphipods (6 species), barnacles, molluscs, ascidians, worms, and other groups. Communities are exposed to three levels of continuous chlorination applied as sodium hypochlorite at these levels: 0.125, 0.250, and 0.500 ppm.

Statistical analyses in 1977 revealed no significant variation within replicate samples (8) at the control level, or within the three chlorination levels. However, statistically significant variance occurred between the control and chlorination levels in the majority but not all tests. The degree of this variance was unexpected because degradation of chlorination or other oxidants was generally believed to occur rapidly in silt-laden estuarine waters.

In addition, the amperometric titrator, an analytical instrument used to detect (and consequently regulate) chlorination levels, failed to register at any chlorination level tested. This evidence suggested that degradation products from chlorination or other strong oxidants may be more significant than the oxidant itself.

Oysters never settled in any "sublethal" concentrations of chlorination, but were found in small numbers in con-

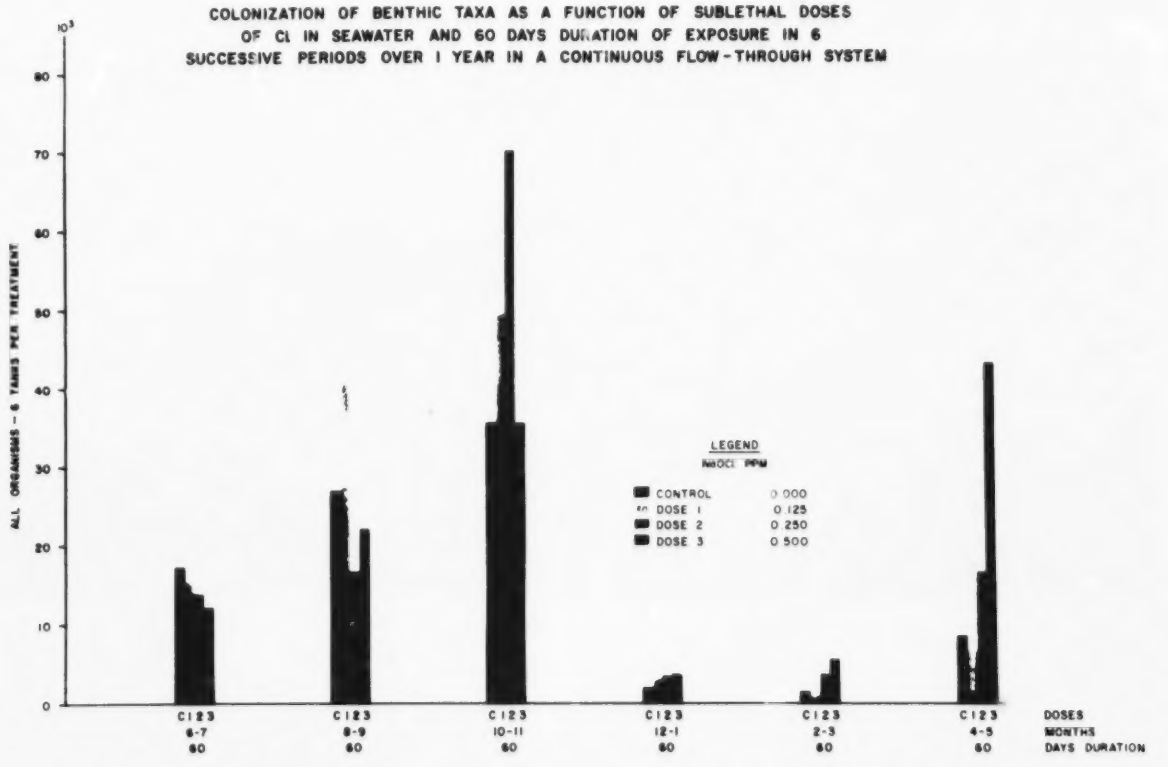


Figure 20. Colonization of benthic taxa as a function of sublethal doses of chlorine is observed in seawater for 60 days in six successive test periods.

and habitats are colonized by many of the organisms in METU/test units. Investigations of life history patterns, seasonal changes of benthic community structures, and trophic interrelationships among marine species contribute to the data base of ecosystem dynamics at Bears Bluff. Investigations in 1978 will focus on algal studies, amphipods, and molluscs. In 1977, the spawning substrate of the most common estuarine fish, the mummichog (Fundulus heteroclitus), was identified; a related fish species (F. majalis) is being studied to elucidate egg-laying cycles hatching of larvae.

Rivulus marmoratus: An Investigation of its Potential as a Cancer Research and Chemical Carcinogen Screening Organism

C.C. KOENIG, Principal Investigator. EPA Grant R805469, Grice Marine Biological Laboratory, College of Charleston, Charleston, SC; W.P. DAVIS, Project Officer

A system using a self-fertilizing, hermaphroditic marine fish (Rivulus marmoratus) to screen chemical substances for teratogenic and mutagenic effects is being used to evaluate compounds identified as EPA testing priorities and as chlorination by-products. The system developed under an IPA agreement administered by the Bears Bluff Field Station will be used in 1978 at the Grice Marine Biological Laboratory. Future experiments will examine use of this species to monitor teratogenic/mutagenic response.

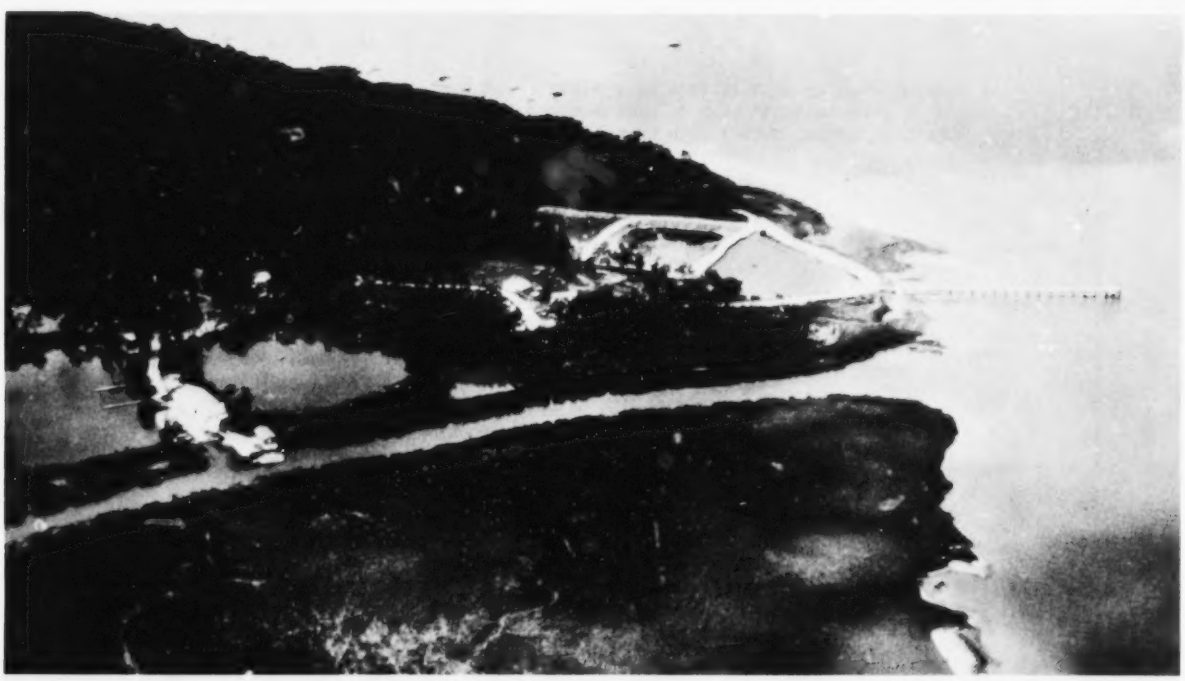


Figure 21. ERL,GB's Bears Bluff Field Station is located on the western side of Wadmalow Island, South Carolina, where estuarine animals are available for tests to determine the impact of biocides on estuarine ecosystems.

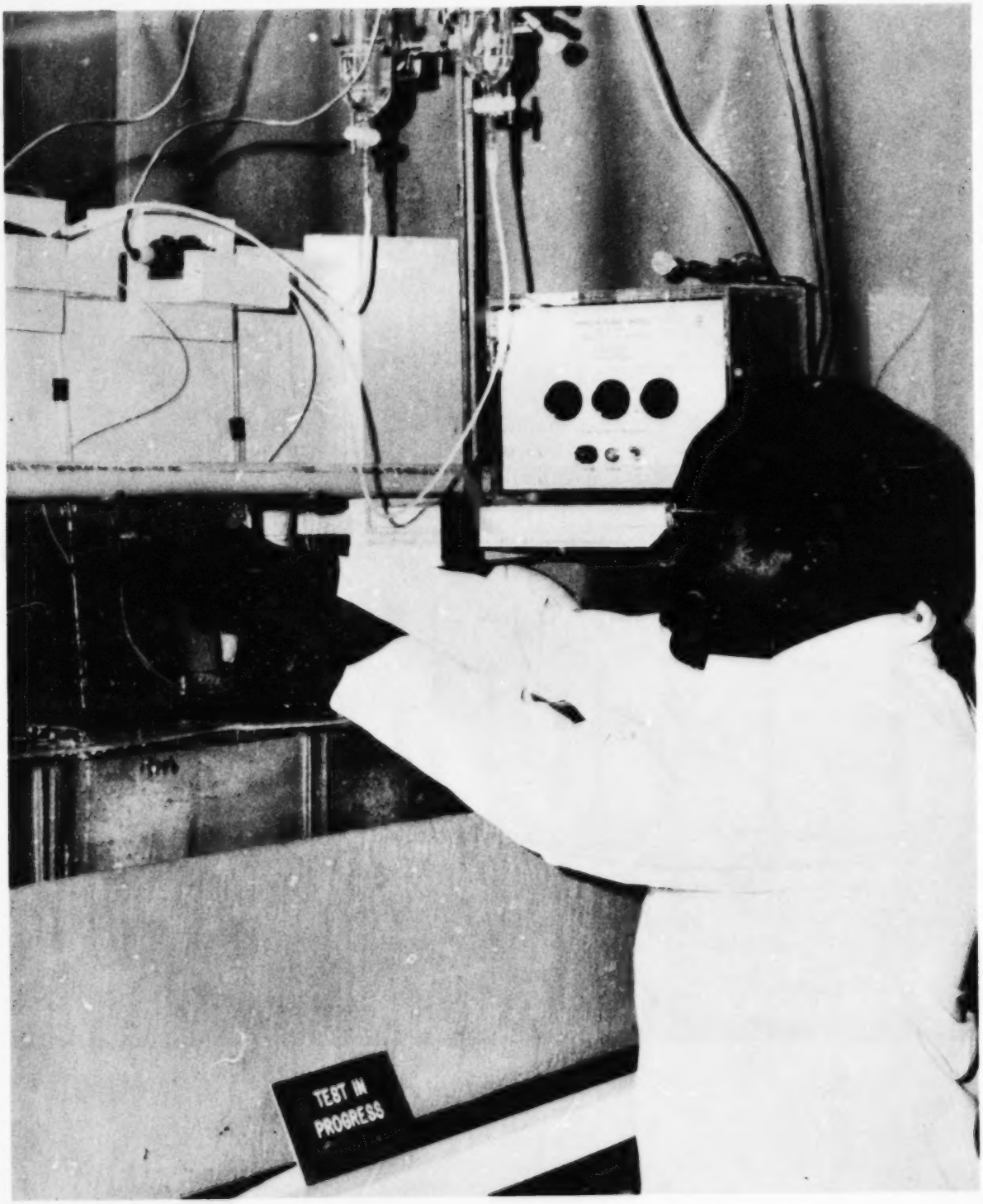


Figure 7. Biological Laboratory Technician Edward Matthews calibrates a Lambda[R] pump for 96-h acute toxicity tests in ERL,GB's aquatic toxicological laboratory. Tests expose mysids and anthropods to toxicants in salinity-controlled, flowing seawater.

Figure 22. Stage I, a U.S. Navy diving platform, serves as an offshore laboratory for ERL,GB marine research on effects of oil-drilling fluids used in oil explorations in the Gulf of Mexico

ENVIRONMENTAL EFFECTS OF OFFSHORE DRILLING

ERL,GB Associate Director, NORMAN L. RICHARDS, Coordinator

Environmental questions regarding the potential impact of chemicals used in developing offshore oil resources are under study in an extramural ERL,GB research program supported by EPA's Office of Energy, Minerals, and Industry. Oil-related research funded under grants and interagency agreements focuses on two major problems: (1) determination of effects of offshore drilling and drilling compounds on the marine environment; (2) detection of drilling emissions believed capable of causing cancer and cell mutations in the marine food web.

New methodology is being developed to predict direct and indirect hazards of drilling and components on marine life indigenous to areas leased for oil exploration. Questions remain unanswered concerning the faculty of fish and crustaceans to retain ingested chemicals and the potential for health hazards of seafood harvested near oil rigs.

The nature of energy-related problems requires a systems approach to their solution. The ERL,GB investigation utilizes a multi-disciplinary research team representing these disciplines: analytical chemistry, statistics, behavioral biology, epidemiology, fisheries biology, toxicology, taxonomy, parhobiology, biochemistry, microbiology, genetics, molecular biology, and physiology.

OFFSHORE OIL AND GAS EXTRACTION

The scarcity of data on the effects of drilling fluids can be attributed in part to their chemical complexity. Analyses of potential effects of chemicals used in well-drilling must take into account variations in mud and cutting compositions due to the type of substrate drilled, drilling depths, availability of mud components, temperatures, and equipment.

Intensified oil explorations in the northeastern Gulf of Mexico has heightened interest in environmental questions regarding the impact of offshore oil development on biological and commercial resources.

During 1977, ERL,GB established an offshore laboratory on a U.S. Navy diving platform in the Gulf of Mexico near Panama City, FL, to study chemicals used in oil drilling (Fig. 22). In an unusual joint research venture involving government and industry, drilling muds for the ERL,GB tests were furnished by Amoco Production Company, which was drilling for oil nearby in the Destin Dome section of the Mafla acreage. In December 1977, Amoco Production plugged and abandoned its hole offshore Florida on the Destin Dome; results of the ERL,GB study with the Destin Dome drilling fluids will be analyzed in the forthcoming reporting period. The project was carried out in conjunction with the American Petroleum Institute's Offshore Operators' Committee under an interagency agreement with the Naval Coastal Systems Laboratory in Panama City.

Effects of Drilling Mud Components

After preliminary screening, pentachlorophenol (PCP) and barium sulfate were selected for intensive study to determine their potential hazards as drilling mud constituents.

The environmental effects of PCP, a pesticide widely used for wood preservation, has been the subject of a number of investigations in recent years. Its versatility has lead to widespread applications of PCP formulations on a global scale.

Although PCP is used as an anti-microbial agent in drilling and packer fluids for offshore oil drilling, little is known concerning the toxicity of PCP to marine and estuarine organisms. Therefore, ERL,GB and the University of West Florida jointly sponsored an international symposium June 27-29, 1977, on Pensacola Beach, FL, to examine available data and recent investigations related to the chemistry, pharmacology, and environmental toxicology of PCP. Proceedings of the PCP symposium were published in Pentachlorophenol, K. Ranga Rao, Ed., Env. Sci. Res. Vol. 12, Plenum Publishing Corp., New York, NY.*

Toxicity studies and behavioral assays with PCP are described earlier as research objectives of ERL,GB's Experimental Environments (pp. 1,10) and Processes and Effects Branches (p. 18). Additional investigations funded by the EPA Office of Energy, Minerals, and Industry are summarized below.

Toxic, Sublethal, and Latent Effects of Selected Petroleum Hydrocarbons and Barium Sulfate on Marine Organisms

K. RANGA RAO, Principal Investigator. EPA Grant R804541, University of West Florida, Pensacola, FL; Norman RICHARDS, Project Officer

Periodic shedding of the exoskeleton is one of the most fascinating phenomenon associated with the growth of crustaceans. Changes in the permeability of the cuticle

*Figures and tables summarizing PCP research in this report are reprinted by permission of the Plenum Publishing Corp.

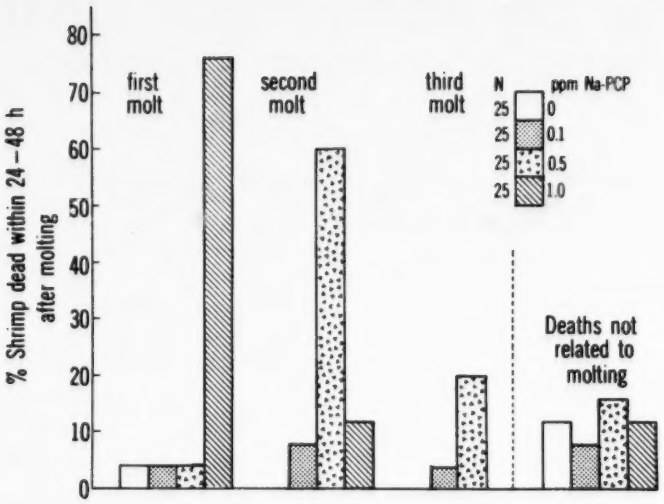


Figure 23. Results of a long-term (66 days) exposure of the grass shrimp, Palaemonetes pugio, to sodium pentachlorophenate (Na-PCP). The incidence of mortalities in relation to ecdysis (molt) is shown.

occur in relation to cyclic shedding, secretion, and hardening of the exoskeleton. Immediately after ecdysis, the new thin cuticle is relatively more permeable and less protective than the thicker, calcified exoskeleton characteristic of the intermolt period. Although earlier literature has not evaluated the toxicity of pesticides to crustaceans at different stages of the molt cycle, previous investigations indicated that newly molted animals exhibit increased sensitivity to toxicants.

This investigation examined the toxicity of Na-PCP to grass shrimp at different stages in the molt cycle.

In 96-h bioassays, the shrimp in later stages of the proecdysial period exhibited a greater sensitivity to Na-PCP than that exhibited by shrimp in the intermolt and early proecdysial stages of the molt cycle. The shrimp in later proecdysial stages generally molted (underwent ecdysis) during the 96-h test period and died shortly after ecdysis. The 96-h LC50 value obtained for these shrimp (0.436 ppm) is the lowest of all the LC50 values reported previously for adult crustaceans and is comparable to those for fish and larval crustaceans. The increased sensitivity to Na-PCP during the early postecdysial period was also apparent in a long-term test (66 days) (Fig. 23).

The observed postecdysial mortality of shrimp exposed to 1.0 ppm Na-PCP was not dependent on the duration of exposure of shrimp to Na-PCP during the proecdysial period. Studies with ^{14}C-PCP indicate that an abrupt increase in the uptake of PCP shortly after ecdysis may cause increased mortalities during this period.

Limb Regeneration

After a limb has been severed from the body of a crustacean, a new limb may grow to replace it. Tests were conducted to determine effects of Na-PCP on limb generation in the grass shrimp throughout the molt cycle.

At predetermined intervals (two or three days), the limb bud sizes of shrimp were measured. Each data point was calculated with a regeneration index (R value):

$$R = \frac{\text{size of limb bud}}{\text{carapace length}} \times 100$$

The R values permitted comparisons of the extent and rate of regeneration among various individuals. The regeneration patterns of 400 shrimp subjected to different treatments revealed that Na-PCP affects the initiation and progress of limb regeneration, such as inhibition of regeneration, delay in limb bud development, or reduction of limb bud growth without altering the intermolt duration. By comparing the R values of control and experimental shrimp on days preceding and following ecdysis, investigators determined the extent (%) of inhibition of regeneration in exposed shrimp. EC50 values were computed by probit analysis. For example, the R values of shrimp 9 days after limb removal yielded the following LC50 values (95% confidence intervals are in parentheses): unfed shrimp, 0.473 ppm Na-PCP (0.306-0.670); fed shrimp, 0.565 ppm (0.452-0.706). EC50 values based on postmolt R values were: unfed shrimp, 0.615 ppm Na-PCP (0.451-0.852); fed shrimp, 0.637 ppm (0.485-0.850). The inhibitory effects of Na-PCP were more pronounced on the initial phases of limb regeneration (involving wound healing, cell division, and dedifferentiation) than on later premolt limb growth (involving further differentiation and cellular enlargement).

These experiments showed that crustacean limb regeneration can be used as a sensitive bioassay for studying effects of environmental pollutants (Fig. 24).

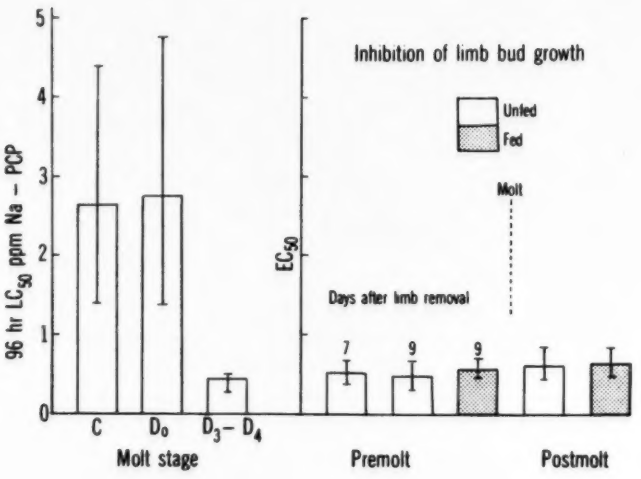


Figure 24. A comparison of the LC50 values (based on 96-h toxicity tests) and EC50 values (based on the extent of inhibition of regenerative limb growth) for grass shrimp, Palaemonetes pugio, exposed to sodium pentachlorophenate. The LC50 values for shrimp are shown at different stages of the molt cycle.

Community Studies

In another phase of the investigation of drilling mud toxicity in 1977, effects of PCP on estuarine benthic communities were studied at ERL,GB in laboratory aquaria fed by a continuous flow of Santa Rosa Sound seawater. The two-stage experiment, concluded March 28, 1977, used meiofauna, the most numerous benthic metazoan found in marine sediments. Meiofauna is defined as an organism that can pass through a 0.5 mm sieve of a width smaller than 0.1 mm. Within the meiofauna grouping, Nematoda, the most common group, was selected as the test species.

In the first phase of the experiment, organisms were exposed to 7, 76, and 622 µg/ℓ concentrations of PCP. Concentrations of 1.8, 15.8, and 161 µg/ℓ PCP were used in the final phase.

Concentrations of 1.8, 7, and 15.8 µg/ℓ PCP did not affect the biomass and density of nematodes. An intermediate concentration of PCP (76 µg/ℓ) caused an increase ($P < 0.01$) in biomass and density of nematodes compared to control aquaria. Higher concentrations of PCP (161 and 622 µg/ℓ) caused a decrease ($P < 0.01$) in biomass and density of nematodes compared to control aquaria. Although species diversity indices of control aquaria did not differ significantly from those of PCP-exposed aquaria, marked changes in nematode species composition and shifts in nematode feeding types were noticed in the aquaria exposed to 161 and 622 µg/ℓ PCP. Nematodes classified as epistrate feeders were most abundant in the control aquaria and aquaria exposed to 1.8, 7, 15.8, and 76 µg/ℓ PCP. Deposit feeders were relatively abundant among the nematodes in aquaria exposed to 161 and 622 µg/ℓ PCP. The alterations in nematodes observed in this investigation appeared to be due to the variations in macrobenthic fauna and food (algae) supply caused by the biocidal effects of PCP and the toxic effects of PCP on meiofauna.

Effects on Respiration

Measurements of oxygen consumption not only indicates metabolic rates of test organisms but also offers an index of stress. Another oil-related research project in 1977 sought to determine effects of Na-PCP and alkyldinitrophenols such as 2,4-dinitrophenol (DNP) on the oxygen consumption of grass shrimp.

Tests showed that oxygen consumption varied in relation to activity at different stages of the molt cycle. Oxygen consumption was measured for extended periods (18 to 24 h) to minimize errors in establishing basal (control) rates of oxygen consumption. In contrast to previously reported progressive increases in oxygen consumption during premolt stages of other crustaceans, oxygen consumption increased significantly prior to and during the shedding of exoskeleton (ecdysis) in grass shrimp. Effects of Na-PCP on oxygen consumption by shrimp varied with the stage of the molt cycle, the concentration of Na-PCP, and the extent of Na-PCP pre-exposure. At concentrations of 1.5 and 5.0 ppm, Na-PCP did not alter the oxygen con-

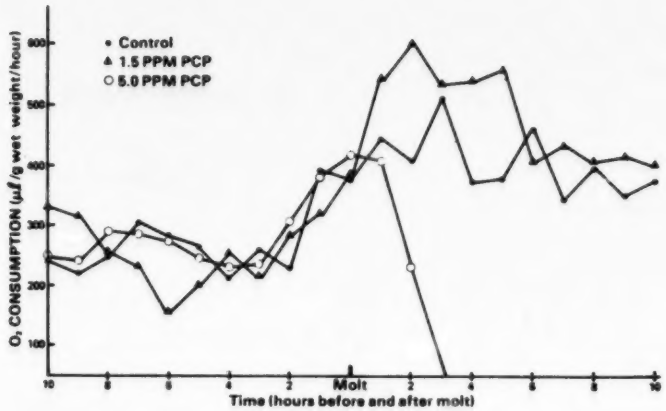


Figure 25. Effect of Na-PCP on the oxygen consumption in Palaemonetes pugio preceding, during, and after ecdysis. Late proecdysial shrimp (Stages D_3-D_4) were used and a basal rate was determined prior to addition of Na-PCP to the medium. Each curve is based on data from a representative shrimp from each group. Shrimp were exposed to 1.5 ppm Na-PCP (triangles) and 5 ppm Na-PCP (open circles).

sumption of intermolt and premolt shrimp. Late premolt shrimp exposed to 5.0 ppm Na-PCP exhibited an increase in oxygen consumption in relation to ecdysis at the same level as consumed by control shrimp. However, after ecdysis, shrimp exposed to 5.0 ppm Na-PCP exhibited a dramatic decline in oxygen consumption and died within 3 h (Fig. 25). Increased sensitivity during the early post-molt period appeared to be related to an increase in the uptake of PCP at this stage, compared to intermolt and premolt stages. A decline in oxygen consumption (as noted above) could be induced in intermolt shrimp by using higher concentration of Na-PCP. Exposure of shrimp to 10 to 20 ppm Na-PCP, or to 5 ppm followed by 20 ppm Na-PCP, caused an initial increase in oxygen consumption and a subsequent decline leading to death. Survival time of intermolt shrimp pretreated with 5 ppm Na-PCP was longer than that of shrimp exposed directly to 10 or 20 ppm Na-PCP. Although 20 ppm DNP (2,4-dinitrophenol) caused an initial increase in oxygen consumption in intermolt shrimp, there was no subsequent decline in oxygen consumption or death during a 24-h exposure.

Effects of Na-PCP and DNP on tissue respiration in vitro also were studied with the blue crab (Callinectes sapidus). At concentrations of 1×10^{-6} M and 5×10^{-5} M, these compounds did not alter the oxygen consumption of the muscle, gill, or hepatopancreas. At a concentration of 5×10^{-3} M, both Na-PCP and DNP caused an inhibition of oxygen consumption of isolated tissues.

In summary, the results of this investigation indicate that the biocidal effects of PCP may not be solely due to its ability to uncouple oxidative phosphorylation but also due to a disruption of the overall metabolic activity.

Effects of Hepatic Enzymes

Further tests with the blue crab in 1977 evaluated effects on Na-PCP in vivo and in vitro on certain hepatopancreatic enzymes. Crabs were maintained in 300 milliosmole seawater at 20°C under controlled conditions.

The hepatopancreas of intermolt crabs were removed and washed in 0.25 M sucrose. The tissue was weighed, homogenized, and fractionated; mitochondrial and microsomal pellets were resuspended in 0.25 M sucrose, divided into aliquots, and kept frozen (-40°C) until needed. The soluble fraction also was divided into aliquots and frozen. The microsomal fraction was used within one week after preparation and other fractions were kept no longer than three weeks prior to assays.

The experiments revealed that Na-PCP had a stronger inhibitory effect than DNP on mitochondrial enzyme from the blue crab hepatopancreas (Fig. 25). However, the enzyme in the cytoplasmic (soluble) fraction was inhibited to a lesser extent in Na-PCP than by DNP.

Calcium-activated ATPase from the microsomal fraction of the hepatopancreas was inhibited by Na-PCP and DNP, both in vivo and in vitro. (Inhibitory effects were more pronounced in vitro than in vivo.) Fumarase (Fum), malate dehydrogenase (MDH), and succinate dehydrogenase (SDH) were inhibited by Na-PCP and DNP in vivo, whereas isocitrate dehydrogenase (IDH) was stimulated. Lactic dehydrogenase was the least affected cytoplasmic (soluble) enzyme in vivo; glutamate-pyruvate transaminase was inhibited to the greatest extent. The fumarase in the cytoplasmic fraction was inhibited to a lesser extent than that in the mitochondrial fraction. The MDH in the cytoplasmic fraction was inhibited to a greater extent than

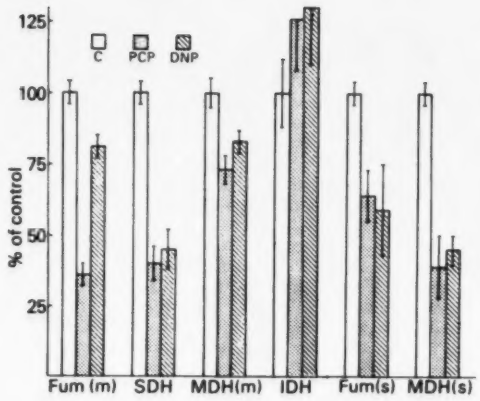


Figure 26. In vivo effect of Na-PCP and DNP on Tricarboxylic Acid Cycle (TCA) enzymes of the blue crab

that in the mitochondrial fraction. Pyruvate kinase and glucose-6-phosphate dehydrogenase were both inhibited 50% by Na-PCP. Na-PCP and DNP had an inhibitory effect on the various enzymes tested in vitro at concentrations of 10^{-4} M or higher (Table 18).

Although considerable work has been completed on the relationship between PCP and ATPase, no definite mechanism of action has been found. A number of questions remain unanswered: Is the effect generalized when the phenol is bound nonspecifically to membrane proteins? Or, is it more specific in its interaction with each enzyme involved? Further investigation is required, but it appears that the uncoupling of oxidative phosphorylation is not the sole basis for the toxicity of PCP.

Ultrastructural Changes

In the final study conducted in 1977 under EPA Grant R804541, ultrastructural changes in the gills of grass shrimp exposed to 1.0 ppm Na-PCP were examined.

Intermolt (stage C) grass shrimp were exposed to 1.0 ppm Na-PCP for the duration of a molt cycle. Gills, hepatopancreas, midgut (portion of the digestive tract surrounded by hepatopancreas), and hindgut (portion of the digestive tract in the abdomen) from control and experimental shrimp at known stages of the molt cycle were observed at the ultrastructural level.

Although signs of pathology were evident in animals in late premolt, extensive pathological changes were not evident until after ecdysis. The extent of pathological changes varied with the tissue examined and the interval between ecdysis and the time of fixation for electron microscopy.

In addition to mitochondrial swelling, the following ultrastructural changes were seen in the gill epithelium of shrimp exposed to Na-PCP (Fig. 27): formation of fluid-filled invaginations of the intermicrovillar apical membrane, increase in lysosomal activity and eventual cytoplasmic and nuclear degeneration. The podocytes in the gill axis, the granular cells, secretory cells, and tegumental gland cells also exhibited mitochondrial swelling, nuclear pyknosis, and eventual cytoplasmic degeneration.

The cells lining the lumen of the midgut and hindgut of shrimp exposed to Na-PCP exhibited swelling of the apical membrane often accompanied by rupture, loss of microvilli from apical foci, and increased lysosomal activity.

Pathological changes noted in the hepatopancreatic cells of the experimental shrimp were: high amplitude swelling of mitochondria including vesiculation of cristae, presence of myelin bodies within mitochondria and rough endoplasmic reticulum, increase of autophagic activity, and loss of microvilli.

The simultaneous deterioration of the midgut, hindgut, hepatopancreas, and gill tissues of shrimp exposed to Na-PCP indicates that focal cell death in any of these regions was not the sole perpetrator of the eventual death of test organisms.

Effects of Drilling Fluids and Oil on Corals Occupying Hard-bank Communities

THOMAS BRIGHT, Principal Investigator. EPA Grant R805441. Texas A&M Research Foundation, College Station, TX; NORMAN RICHARDS, Principal Investigator

Coral reefs represent an important resource in the Gulf of Mexico. This research grant will address three ques-

Table 18. Effect of Na-PCP and DNP on Calcium Activated ATPase in vivo

	Control	Experimental	
Sample	seawater	DNP	Na-PCP
Conc.	- - - -	6 µg/g	6 µg/g
Activity	0.339 ± 0.012	0.322 ± 0.007	0.279 ± 0.011
% Activity	100%	95%	82%

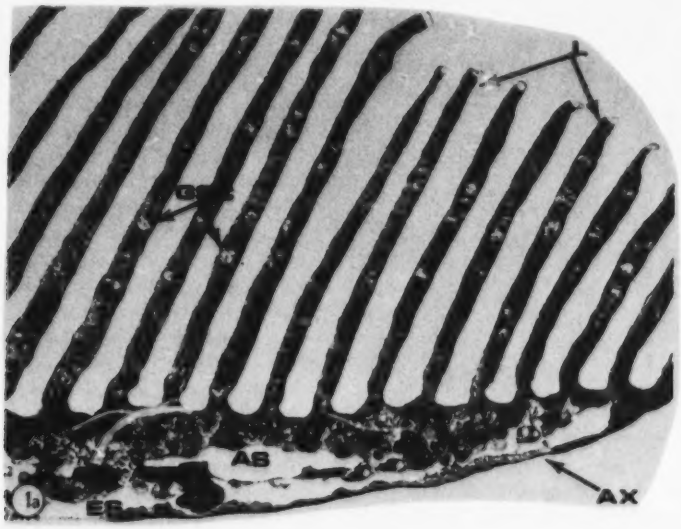


Figure 27. Light micrograph of sagittal section of a pleurobranchiate gill of Palaemonetes pugio showing the association of lamella (L) with the main axis (AX). Note the affarent (AS) and efferent (ES) hemolymph sinuses and the close association between the clear gland (CG) and efferent sinus. Note also the reticulate glands (RG) and the granular secretory cells (GSC).

tions: (1) Can oil drilling operations undertaken in the vicinity of coral reefs adversely affect their structure? (2) Is it feasible to shunt cuttings and drilling fluids? (3) Should discharges from oil drilling activities be removed by barge?

In 1977, corals were collected and exposed to drilling muds at ERL,GB. An evaluation of these exposures will be completed in the next reporting period.

FUTURE GOALS

Policy decisions on the use of drilling fluids currently are based on static, 96-h LC50 determinations, observations of divers, and theoretical models of pollutant dispersion. Research on drilling-fluid constituents was initiated at ERL,GB to provide a better data base to predict the relative biological and human health hazards of drilling fluids and to develop more relevant laboratory methods for xenobiotic evaluation.

Results to date indicate that drilling-mud constituents may affect the structure and function of ecosystems, both directly and indirectly. Improved techniques are needed to determine the potential for these substances to bioaccumulate and to contaminate organisms indigenous to areas under lease for oil drilling operations. In addition, further toxicological information is required to assess alternative chemicals available for offshore oil production.

MARINE FOOD CHAINS

The second phase of ERL,GB research on energy sources is concerned with cancer-causing properties of shale-oil fuels and their potential threat to fish caught for the marketplace.

This program will investigate carcinogens found in shale-oil fuels, their metabolic fate in the marine food chain, their persistence in the environment, and their potential for accumulation in seafoods (Fig. 28).

Research in 1977 examined the potential for uptake, transfer, and depuration of polynuclear aromatic hydrocarbons (benzo[a] pyrene and chrysene) by representative members of a simulated food web. Tests showed that chrysene is accumulated by protozoa, algae, mysids, and polychaete worms. Spot bioaccumulated detectable concentrations of chrysene from exposed mysids; chrysene was detected only in the liver of mangrove snapper that consumed fish exposed to chrysene in water.

In further studies, an algae-oyster food chain was found to accumulate only a small amount of chrysene or benzo[a] pyrene. Lugworms did not depurate chrysene with the rapidity observed in oysters. Related studies funded under this program are described earlier (Grants R804458, p. 26).

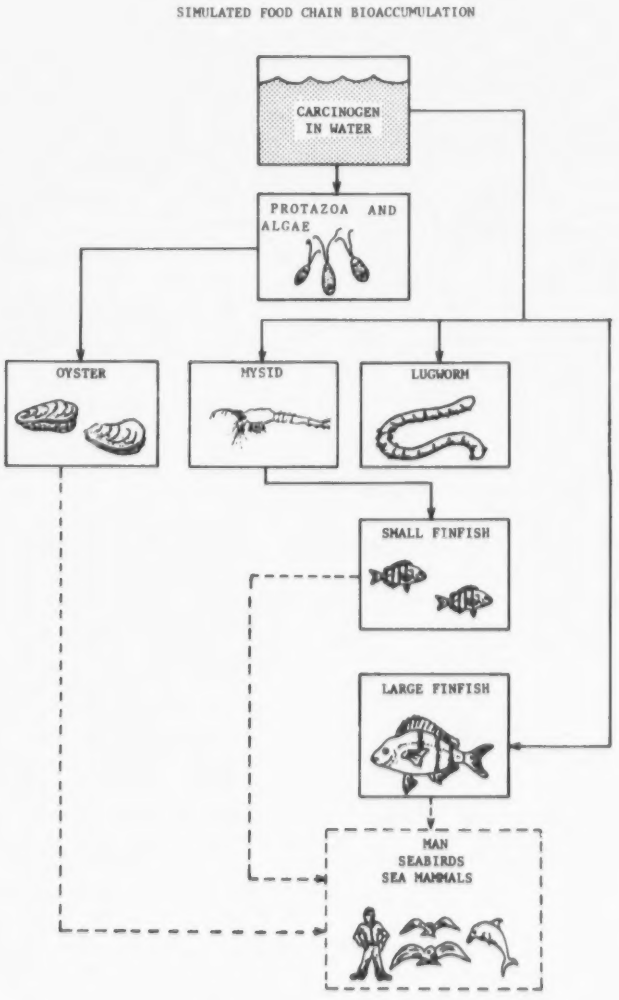


Figure 28. Simulated aquatic food web

Carcinogenic Photooxidation Product from Petroleum PAH's at Air-Sea Interface

J.L. LASETER, Principal Investigator. EPA Grant R804647. University of New Orleans, LA; NORMAN RICHARDS, Project Officer

The research objective is to determine major photooxidation products of petroleum derived polynuclear aromatic hydrocarbons (PAH). Studies will attempt to partially elucidate "weathering" phenomenon of petroleum at an air-seawater interface. Many major oxidation products were identified in 1977, and representative compounds were selected from field samples for detailed study. Structure of these compounds will be confirmed by chemical synthesis. Numerous aromatic hydrocarbons and their biologically significant oxidation products have been supplied to the project officer and will be screened for mutagenic properties.

Accumulation/Elimination of a Certain Aromatic Petroleum Hydrocarbon

ROBERT FARRAGUT, Principal Investigator. Interagency Agreement IPE-IAG-D6-0084. National Oceanic and Atmospheric Administration, Miami, FL; NORMAN RICHARDS, Project Officer

Selected marine species commonly consumed by man were tested to determine how they accumulate and eliminate carcinogenic aromatic petroleum hydrocarbons. Snapper and shrimp were observed in separate control tanks and tanks containing various concentrations of test compounds. After exposures, the cephalothorax, gut, and tail of shrimp and the liver, gall bladder, gut, and flesh of snapper were analyzed.

The mangrove snapper, also known as the grey snapper (Lutjanus griseus), was exposed to 5 µg/ℓ chrysene in 1690-ℓ tanks. Analysis did not reveal any detectable levels in snapper flesh, but chrysene was detected in the liver. Shrimp data will be analyzed in future work.

Subcellular Distribution of Enzymes of the Marine Ciliate (Parauronema acutum)

DONALD G. LINDMARK, Principal Investigator. EPA Grant R805364010, the Rockefeller University, New York, NY; NORMAN RICHARDS, Project Officer

Ensymatic capabilities of marine protozoa to transform or bioaccumulate polynuclear aromatic hydrocarbons were investigated in another 1977 study related to the marine food web.

The following enzyme activities were detected in homogenates of the symbiote-free marine ciliate, Parauronema acutum (mU/mg protein): malate dehydrogenase (6000), NAD(P): acceptor oxidoreductase (dichlorophenol indophenol, 75), isocitrate dehydrogenase (93), non-specific esterase (p-nitrophenol acetate, 3), and the following acid hydrolases: acid phosphatase (300), -galactosidase (14), -glucosidase (6), and proteinase (urea denatured hemoglobin, 200). Lactate, alcohol, NAD(P) dehydrogenase, and malate dehydrogenase (decarboxylating) activities could not be demonstrated.

Differential centrifugation demonstrated the sedimentable nature of all enzymes except esterase. Malate dehydrogenase and NAD(P): acceptor oxidoreductase possibly localized in the mitochondria, and isocitrate dehydrogenase possibly localized in peroxisomes sediment at 2500 rpm for 10 min and in hydrolase sediment at 10,000 rpm for 30 min, suggesting localization in a separate particle population.

All particle-associated enzymes exhibited structure-linked latency (50-80%), but lost latency and became non-sedimentable after Triton X-100 treatment or freezing and thawing (three times). The only exception is an acid phosphatase which remains sedimentable after freezing and thawing. These data suggest the occurrence of lysosome-like organelles in this ciliate and also give biochemical evidence for the occurrence of mitochondria and peroxisomes.

Detection of Carcinogens in Seawater; Use of Hybrid Fish and Food Chains

DOUGLAS HUMM, Principal Investigator. EPA Grant R804650. University of North Carolina, Chapel Hill, NC; NORMAN RICHARDS, Project Officer

A sensitive in vivo method for the detection of carcinogens in hybridized fish is under study. The investigator has produced several hybrid fish that appear to be appropriate for extensive validation of the methodology. Results will be published after the histopathology is complete.

A tentative agenda has been established for a symposium on "Mutagenic, Carcinogenic, and Teratogenic PNA Hydrocarbons in the Marine Environment" in the summer of 1978 at ERL,GB. Patricipants will report current research on physical and chemical fate; methods for concentration, separation and detection; the pharmacodynamics of activation, detoxification; accumulation and depuration; fate in marine trophic systems; the genetic basis for in vivo screening methods; quick screen and prescreen methods; laboratory and field observations on tumor formation. The overall objective will be to assess the potential of marine animals as models for carcinogenesis, their use for monitoring the aquatic environment, and the potential risk to human health from the consumption of seafoods contaminated with man-mobilized hydrocarbons.

GRANTS AWARDED IN 1977

Investigation of Enzymatic Screening Tests for Mutagens in Environmental Pollutants from Synfuel Operations

JOSEF SCHMIDT-COLLERUS, Principal Investigator. EPA Grant R805671. University of Denver, Denver, CO; NORMAN RICHARDS, Project Officer

An in vitro test using the inhibition of 4-biphenyl hydroxylase activity will be developed to determine presence of mutagenic compounds.

Novel Techniques for the Concentration and Separation of Toxic Substances from Estuarine Waters

ELIAS KLEIN, Project Manager. EPA Grant R805656. Gulf South Research Institute, Baton Rouge, LA; NORMAN RICHARDS, Project Officer

Methods to concentrate, separate, and detect compounds with toxic and mutagenic properties in estuarine waters will be developed. Techniques, such as affinity chromatography, reverse osmosis, and Donnan dialysis will be used to test isolated substances for mutagenic properties.

Genetic Variation and Resistance to Carcinogens in Natural Waters

R. JACK SCHULTZ, Project Manager. EPA Grant R805195. University of Connecticut, Storrs, CT; NORMAN RICHARDS, Project Officer

The study will evaluate feasibility of using isogenic fish as carcinogen bioassay organisms. The validated bioassay system would be used to test the carcinogenic properties of compounds. The importance of genetic variability of test organisms also will be determined.

Susceptibility of Genetically Defined Stocks of Fish to Chemical Carcinogens

KLAUS KALLMAN, Project Manager. EPA Grant R805389. Osborn Laboratories of Marine Sciences, Brooklyn, NY; NORMAN RICHARDS, Project Officer

The genetic structure of fish was investigated in 1977 in relation to their susceptibility to polycyclic aromatic hydrocarbon carcinogenesis. Genetically defined fish with the following properties were under study: inbred homozygous from natural populations, inbred homozygous from laboratory stock, heterozygous with coadapted gene pools, heterozygous with poorly adapted gene pools.

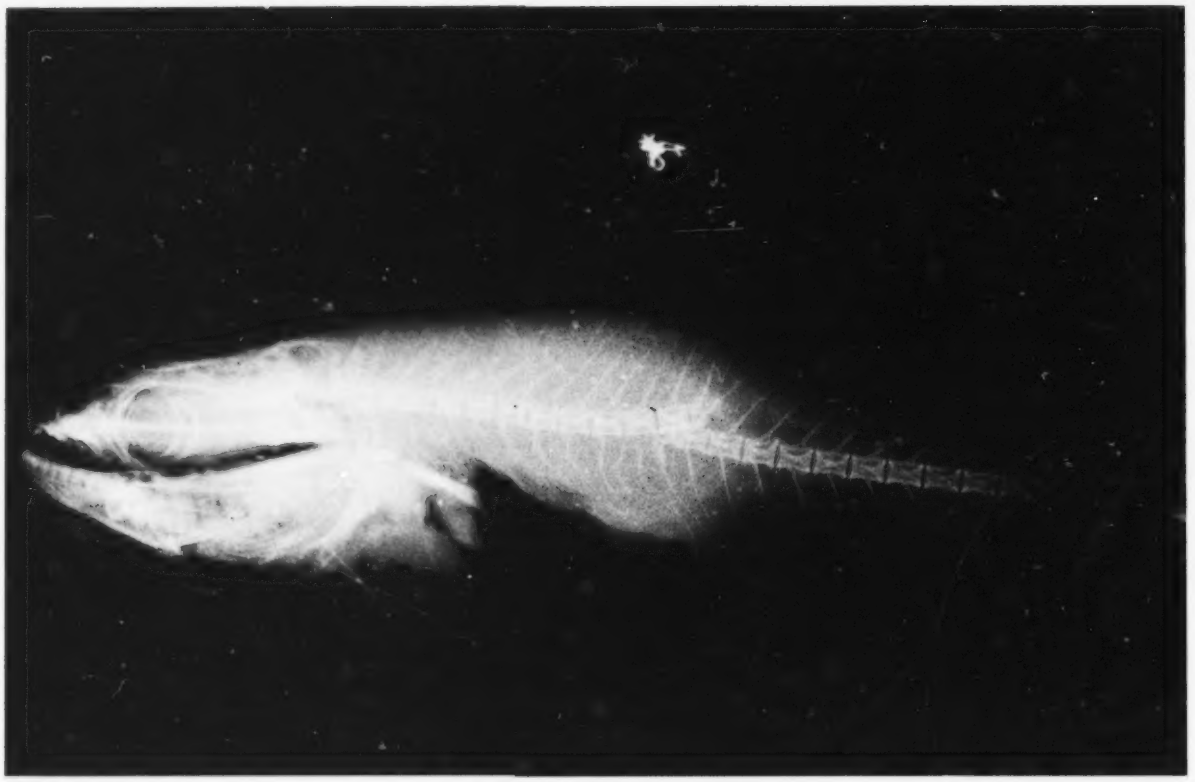


Figure 29. Radiograph shows blue fish collected in the James River fish kill (1974). The broken vertebral column is similar to condition induced in laboratory tests of Kepone. (See p. .)

KEPONE

ERL,GB Deputy Director, T.T. DAVIES, Coordinator

Kepone, a pesticide discovered in the James River Estuary in 1975, was found to be highly toxic to marine animals, including crabs, fish, and oysters. Concentrations of Kepone considered unsafe for human consumption have been measured in tests of commercial marine species indigenous to the James River/Chesapeake Bay.

In response to health and the environmental hazards posed by the contamination of the James River, Congress directed EPA in 1976 to study the effects of toxics in the Chesapeake Bay ecosystem. ERL,GB has assumed an active role in the continuing scientific assessment of damage caused by Kepone and its accumulation in the James River Estuary.

Research coordinated by ERL,GB during this reporting period focused on the routes and rate of the insecticide's transport and the fate of Kepone bound to estuarine sediments. A major objective is to determine how long Kepone will persist in bottom sediments of the James River/Chesapeake Bay and to project the time required to reduce Kepone residues to safe, acceptable concentrations. The answer to this question will assist the State of Virginia and federal authorities in formulating plans to preserve the health and productivity of the estuary.

An investigation funded under an EPA grant with the Virginia Institute of Marine Science (R804993) found high Kepone concentrations in the biota of the James River Estuary. Robert Huggett, project manager, reported that concentrations in edible tissues of most fresh and estuarine fin- and shellfish commonly ranged from 0.1 to more than 1 mg/kg. Kepone concentrations were shown to increase in anadromous fish in relation to the time spent in the river.

Studies conducted thus far show that Kepone does not degrade either biologically or chemically in simulated estuarine systems, indicating that normal degradation processes probably will not alter existing concentrations of Kepone in James River water and sediment.

Laboratory tests at ERL,GB have demonstrated that Kepone concentrations >0.008 µg/ℓ in water cause observable, detrimental effects on crabs, shrimp, or fish; a concentration of 0.15 mg/kg Kepone in tissues of oysters fed to blue crabs had a deleterious effect on the crabs.

Field-collected, egg-bearing grass shrimp, recently hatched larvae, eggs, and newly hatched larvae, and laboratory-reared postlarvae, showed variations in Kepone concentrations ranging from undetectable to levels of 0.6 ppm. Populations from the James River and the nearby Lafayette River showed the highest concentrations of Kepone; distant populations showed lower levels. Laboratory-reared postlarvae (representing all six populations) had very low or non-detectable Kepone concentrations.

Two long-term studies were conducted to determine the uptake and depuration of Kepone in blue crabs (Callinectes sp.). In the first, Kepone was administered to crabs in seawater (0.03 or 0.3 µg/ℓ) or in food (eastern oyster, Crassostrea virginica, containing 0.25 µg/g Kepone). Results indicate that uptake of Kepone in 28 days was primarily through the contaminated oysters. When these crabs were supplied with Kepone-free seawater and uncontaminated oysters for 28 days, no depuration of the insecticide was evident. There were indications of adverse effects in crabs fed oysters that contained 0.25 µg/ℓ Kepone.

Results of the second study indicated that blue crabs fed Kepone-contaminated oysters, followed by a diet of Kepone-free oysters for 80 days, had detectable concentrations of the insecticide in their muscle and remaining tissues. Blue crabs that ate oysters containing Kepone in concentrations similar to those in food of crabs from the James River died or molted less frequently than crabs fed Kepone-free oyster meats. In addition, long-term exposures of sheepshead minnows and mysid shrimp to Kepone concentrations less than 0.08 µg/ℓ reduced survival, reproduction, or growth.

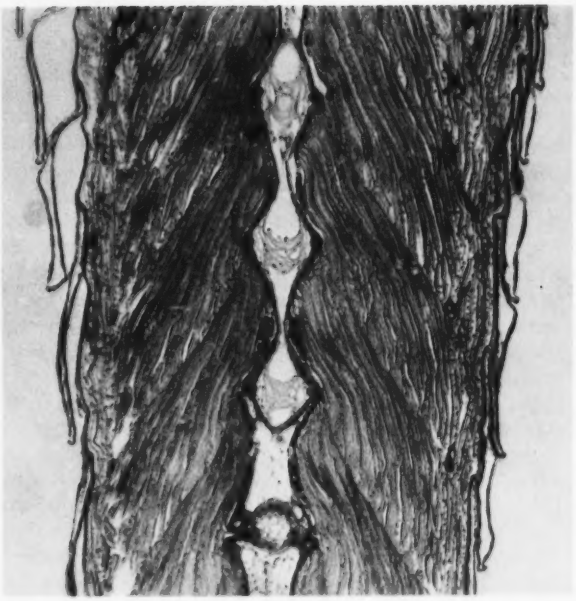
In another series of tests, Kepone-induced scoliosis, or lateral curvature of the spine, in sheepshead minnows exposed to a relatively low concentration of the organochlorine. This syndrome is caused by diverse agents that possibly act on the central nervous system. Effects associated with scoliosis in the sheepshead minnows included: disruption of myotomal patterns, inter- and intramuscular hemorrhage, fractured centra of vertebrae, and death (Figs. 30, 31, p.54).

Bluefish exposed to Kepone in ERL,GB laboratory tests exhibited a broken-back syndrome identical to that observed in several species of fish examined after the mass kills in the James River in 1973 and 1974 (Fig. 29).

In addition, nonlinear statistical models were developed in 1977 to describe the uptake and depuration of pesticides. The models described biological data as a single equation, thus allowing variations due to many physical, chemical, biological, and random error factors to be analyzed simultaneously.

Under EPA Grant R804563, Donald J. O'Connor and Kevin J. Farley, of Manhatten College, the Bronx, New York, will develop a model for evaluating time required to reduce Kepone to harmless concentrations. Phenomena related to the transfer of Kepone from its initial discharge point (Hopewell, VA) to fishery stock will be represented in the model.

ERL,GB research findings on the effects of Kepone has been available to the state of Virginia and federal authorities who are attempting to minimize the future impact of this insecticide on the environment and human health.



Figures 30 and 31. A normal backbone of sheepshead minnow (left) can be compared to broken backbone (right) induced by Kepone in laboratory tests with the sheepshead minnow.

ENVIRONMENTAL PATHOBIOLOGY

J.A. COUCH, Coordinator

Environmental hazards attributed to toxic substances range from mild temporary dysfunction of organisms and ecosystems to acute disorders and death. Some toxic substances are known or potential carcinogens, mutagens, or teratogens. ERL,GB's Environmental Pathobiology Team investigates sublethal effects of pesticides and pollutants that may cause neoplasms (tumors) in aquatic species. The movement of carcinogens and mutagens through the marine food web is also under study.

An understanding of the effects and behavior of these toxicants will enable scientists to better relate environmental phenomena to possible risks to human health, particularly in regard to cancer-causing agents.

Pesticide-related Studies

Effects of insecticides, herbicides, and fungicides on aquatic species are studied from subcellular to higher population levels to determine the nature and degree of damage caused by exposure to pollutants. In 1977, the Environmental Pathobiology Team examined and described major tissue and cellular changes in sheepshead minnows exposed to the insecticide Kepone and the herbicide trifluralin.

Kepone exposure (1 to 5 µg/ℓ) resulted in broken backs in sheepshead minnows (Figs. 30, 31). Vertebral damage was severe enough to cause death of the exposed fish. Massive fish kills were found in the James River, Virginia, in 1973 and 1974. Many of the dead or dying fish recovered in these kills had broken backs (Fig. 29), thus indicating a possible relationship between the mass mortalities of fish and the syndrome of broken-backs in Kepone-exposed fish in the laboratory.

Sheepshead minnows exposed to trifluralin from zygote to juvenile stages developed a vertebral disorder. Vertebral columns of young fish exposed to 5.5 to 31 µg/ℓ trifluralin for 28 to 51 days were 5 to 100 times their normal size (Fig. 32). The cause of these enlarged vertebrae was attributed to hypertrophy of the boney tissue in the vertebral wall. This condition was unprecedented in animals previously exposed to chemicals. Fish suffering from the disorder of vertebrae are not able to swim or compete successfully for food or mates.

Future research will be conducted on the mechanism of trifluralin-induced dysplasia in fishes.

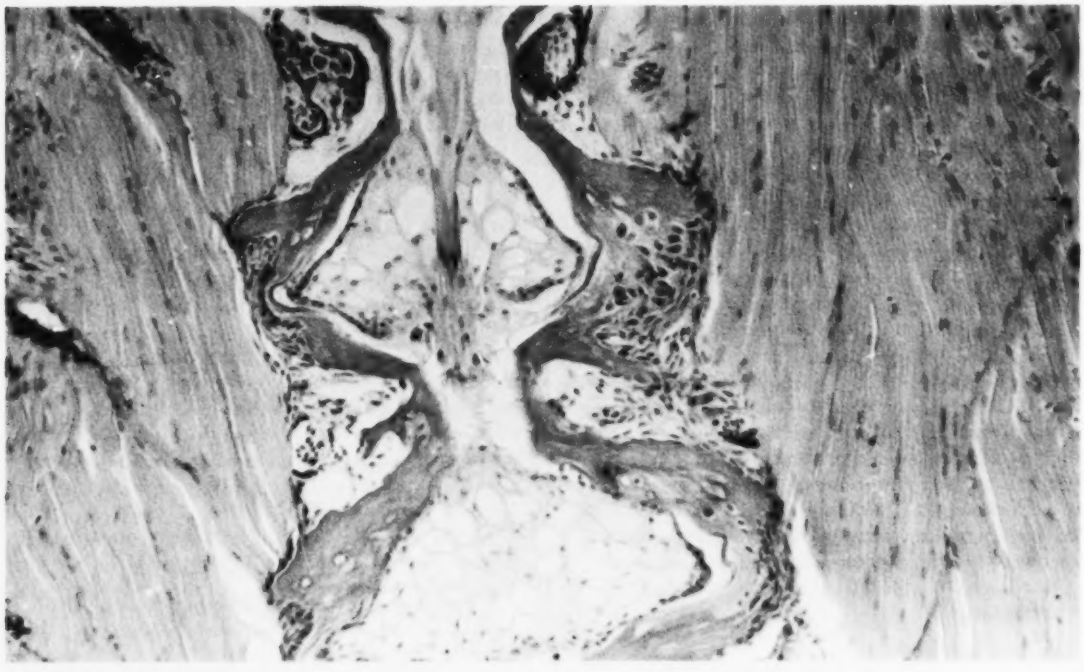
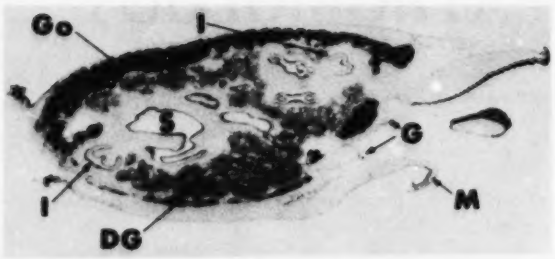


Figure 32. A backbone from young sheepshead minnow exposed to the herbicide trifluralin shows tremendous hyperplasia of the vertebral column. Normal backbone is illustrated in preceeding figure.



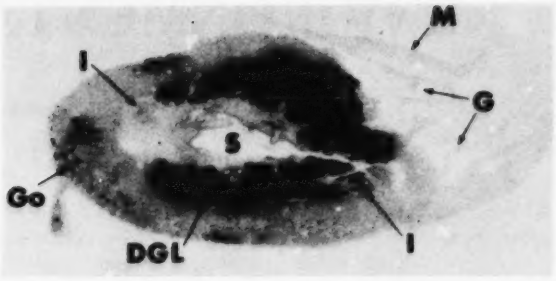


Figure 33. Section from the American oyster illustrates its general morphology: I, intestinal loops; G. gills; DG, digestive glands.

Figure 34. Radioautograph shows 9 sections of an oyster exposed to radioactive labeled benzo[a] pyrene for 7 days. Radioactivity is concentrated in the digestive gland (DG) and gonad (GO) areas.

Carcinogen Aquatic-animal Model System

The potential use of an estuarine invertebrate in early detection of chemical carcinogens in the environment is under study at ERL,GB. In 1977, oysters were exposed to 1.0 and 5.0 ppb benzo[a] pyrene (BP) and 3-methylcholanthrene (3-MC) eight months to one year or longer. Periodic samples of oysters and water in the exposure system were analyzed for carcinogen concentration, uptake, and accumulation. Oyster tissues also were examined histologically for pathological changes indicative of carcinogenic effects (cellular disorders) (Figs. 33, 34).

Accumulation of BP and 3-MC by oysters appeared to relate to concentrations of these chemicals in exposure water. Oysters exposed for 8 months to 1.0 µg/ℓ BP and 3-MC accumulated no more than 0.264 µg/g carcinogen in their tissues. Oysters exposed for only 3 months to 5.0 µg/ℓ BP and 3-MC accumulated from 0.440 µg/g to 6.470 µg/g carcinogen in their tissues. Algae and sessile bacteria, as well as sessile invertebrates attached to the exposure tanks, probably competed for absorption and adsorption of carcinogen with oysters when the exposure concentration was only 1.0 µg/ℓ. When exposure concentrations were raised to 5.0 µg/ℓ, oysters accumulated carcinogen at a relatively higher rate. This phenomenon indicates that a threshold concentration of polycyclic aromatic hydrocarbons may be needed in estuarine waters before significant bioconcentration occurs in oysters.

To date, histological alterations found in exposed oysters have been incipient and probably indicate inflammatory responses. Future studies will follow the course of cellular alterations in exposed oysters to determine if tumors are induced.

Interactions of Chemical Pollutants and a Virus

Most established host-parasite or host-pathogenic relationships represent more or less balanced interactions of long evolutionary development. Few experimental studies of aquatic ecosystem have examined possible interactions among such environmental factors as pollutants, host species, and parasites or pathogens. Available data, however, indicate an unusual adverse effect on some host aquatic species by certain of their natural parasites when the complex is exposed to pollutants or pollutant mixtures.

This research project investigated the potential use of an aquatic animal, host-virus system developed at ERL,GB as a bioassay tool to measure and predict possible interactions among pollutants, viruses, and their hosts. The system used penaeid shrimp as the host, a shrimp-specific Baculovirus, and selected pollutant chemicals, including polychlorinated biphenyls (PCBs).

Earlier experiments showed that exposure of small groups of shrimp (8 to 35 per group) having a low natural prevalence of Baculovirus to 1 to 3 ppb of PCB (Aroclor^{R} 1254) for 10 to 25 days resulted in an increase in the Baculovirus prevalence. In two of three tests, the exposed shrimp exhibited a higher prevalence and intensity of infection when compared to controls during and after exposure periods.

In a more extensive test involving large numbers of control and experimental shrimp, the enhancement of viral prevalence was validated.

Both exposed and control groups had the same prevalence of patent viral infections at the beginning of this test (Fig. 35). The rather abrupt increase in viral prevalence in exposed shrimp (from 23.3 to 75% in 35 days) probably was related to an undefined interaction of host, chemical stressor (Aroclor 1254), and virus.

Several possible interactions could account for a more rapid increase of viral infections in chemically stressed shrimp than in control shrimp: loss of resistance to new viral infections in shrimp hosts due to toxic effects of Aroclor 1254; enhanced latent or occult viral infections possibly carried by all or most shrimp from enzootic viral populations; increased virulence of virus when exposed in

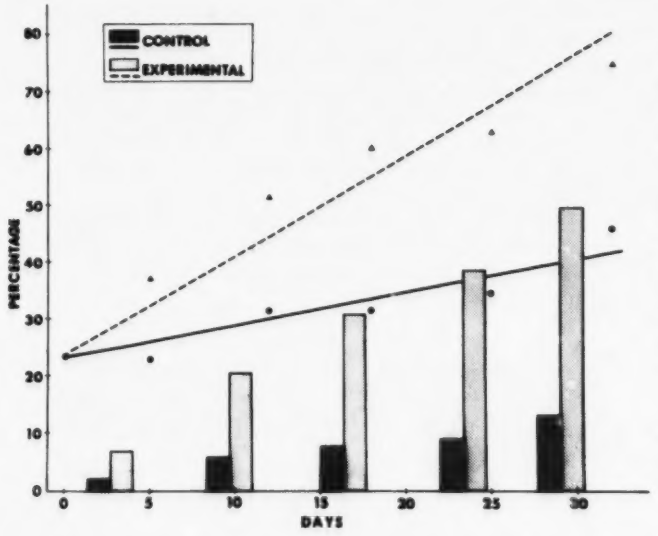


Figure 35. Graph demonstrates viral prevalence and mortality in shrimp samples exposed to Aroclor 1254*

vivo to Aroclor 1254; and greater susceptibility of intoxicated, weakened individuals to cannibalistic habits of lesser-intoxicated exposed shrimp.

Future studies will use the Baculovirus-shrimp system to investigate bioassays with additional chemicals. Tentative criteria for evidence of interaction are: increased viral prevalence in exposed animals; increased intensity of infection; increased mortality; and enhanced cytopathic effects.

Characterization of Shrimp Baculovirus

M.D. SUMMERS, Principal Investigator. EPA Grant R803395, The University of Texas, TX; J.A. COUCH, Project Officer

Baculovirus cytopathology and ultrastructure have been studied extensively and until recently known baculoviruses were observed only in insects. However, Dr. J. A. Couch of ERL,GB discovered a baculovirus in the pink shrimp, Penaeus duorarum, in studies conducted in 1974 and 1975, thus extending the host range of this class of viruses into the class crustacea. Since 1975, reports have cited possible baculovirus infections in shellfish in other areas. Observations of baculoviruses in non-insect arthropods are particularly significant in light of recent use of insect baculoviruses as microbial pesticides for agricultural pest control.

This investigation was undertaken to partially characterize the pink shrimp baculovirus, which was found to be morphologically similar to insect baculoviruses. Research compared biochemical, structural, and biological properties of the shrimp virus to known properties of insect viruses.

Results confirmed earlier evidence that the shrimp and insect nuclear polyhedrosis baculoviruses (NPVs) are structurally related. The project pointed out the need to study host specificity with regard to baculoviruses in more definitive detail. Although current use of existing viral pesticides does not appear to warrant concern, the discovery of marine baculoviruses suggests that a virus restricted in terms of specificity to insects is not absolute as previously thought. Results of this study were published in the EPA Ecological Research Series, Report No. EPA-600/3-77-130, November 1977.

Effects of Kepone on Animals in James Estuary, Virginia

C. SINDERMANN, Principal Investigator. EPA-IAG-D6-0124. National Oceanic and Atmospheric Administration (NOAA); J.A. COUCH, Project Officer

Chromosomal and histopathological abnormalities induced in shellfish by Kepone are under investigation in a three-phase project:

(1) Chromosomal studies in larval offspring of oysters (C. virginica) to determine the degree and type of chromosomal abnormalities in larvae of oysters exposed to Kepone. Larval offspring of unexposed (control) oysters will be compared with larvae whose parents had been exposed for various time periods to varied levels of Kepone.

(2) Histopathological and gametogenic studies on four species of molluscs in Kepone-contaminated and in non-contaminated waters. Organisms (collected seasonally) on a transect from Bailey's Creek, VA, to the mouth of the James River will be examined histopathologically. At least 30 animals per site of the following species will be collected: R. cuneata, C. virginica, M. mercenaria, and M. balthica.

(3) Adult oysters exposed to Kepone under controlled conditions will be shipped to the Milford, Connecticut, laboratory for "conditioning" and spawning. Chromosomal studies will be performed on larval offspring.

Benzo[a]pyrene Metabolism in the American Oyster (Crassostrea virginica)

R.S. ANDERSON, Chief Investigator. EPA Grant R804435. Sloan-Kettering Institute for Cancer Research, D.S. Walker Laboratory, Rye, NY; J.A. COUCH, Project Officer

Susceptibility of aquatic species to carcinogens or mutagens may depend on the ability of these species to me-

*Reprinted by permission from the New York Academy of Sciences, Ann. N. Y. Acad. Sci., Sept. 1977, Vol. 298, p. 502.

tabolize xenobiotics to proximal or active intermediate compounds. This investigation was undertaken to assess the capacity of marine mollusks to metabolize the ubiquitous pollutant benzo[a] pyrene (BP), a carcinogenic polycyclic aromatic hydrocarbon.

The susceptibility of invertebrates to chemical carcinogens is largely unknown. One objective of the study was to determine if the potent mammalian carcinogen BP could be considered a possible bivalve carcinogen. Aside from detrimental effects on the oyster, presence of carcinogenic BP metabolites in fish commonly consumed by man may have public health implications.

A sensitive radioisotopic system was developed to quantify alkali-soluble and water-soluble BP metabolites produced by oyster mono-oxygenase. An NADPH- and O_2-dependent aryl hydrocarbon hydroxylase (AHH) was shown to be located in the digestive glands of oyster bivalves associated with the microsomal subcellular fraction. The specific activity of oyster AHH, considerably lower than that observed in laboratory mice, was consistently demonstrable. Water-soluble derivatives were produced primarily by BP metabolites.

Some indications were found that oyster AHH is induced by chronic exposure to environmental carcinogens BP and 3-methylcholanthrene. Further, evidence suggested that exposure to polychlorinated biphenyls (PCBs) caused AHH induction.

BP metabolites produced by oyster AHH were identified by high-pressure liquid chromatography. The generation of various dihydrodiol, quinone, and hydroxy BP derivatives was shown (production was augmented in PCB-exposed oysters). No evidence was found regarding the production of suspected ultimate carcinogenic BP metabolite (7,8 diol-9, 10-epoxide); the 7,8-diol and the mutagenic 4-5 oxide derivates were present in the oyster. However, all metabolites are not known.

Results of the research conducted from July 1, 1976, to June 30, 1977, are summarized in EPA's Ecological Research Series, Report No. EPA-600/3-78-009, January 1978.

Effects of Petroleum Compounds on Estuarine Fishes

B.J. MARTIN, Principal Investigator. EPA Grant R804527, University of Southern Mississippi;
J.A. COUCH, Project Officer

This investigator will attempt to characterize chemically induced tumors in teleost fishes. Both marine and freshwater species will be exposed to low levels of a known carcinogen for 300 days. Tissue from the liver, kidney, intestine, and gills of fish developing tumors or any pathogenesis will be studied histologically. Tissues from all the fish with no grossly apparent pathogens at the end of the test will also be studied in the same manner in order to detect any neoplasias or preneoplastic conditions.

This research is expected to: (1) provide evidence concerning the quantities of benz[a] pyrene necessary to induce neoplasia; (2) supply additional data concerning tumors in teleosts; (3) establish the feasibility of using teleost fish as early indicators of carcinogenic hazards in the aquatic environment; and (4) demonstrate usefulness of this methodology to screen compounds for carcinogenic properties.

Fish were exposed for several months to different concentrations of BP and 3-MC. Differential toxicity of these compounds to fish have been found and histological studies will be continued.

Studies on Environmental Chemical Carcinogens Present in Economically Important Molluscs and Crustaceans from Oregon Bays, Estuaries, and Inshore Areas

C. MIX, Principal Investigator. EPA Grant R804427, Oregon State University, Corvallis, OR;
J.A. COUCH, Project Officer

These studies focus on: (1) possible incidence of selected environmental carcinogens in economically important molluscs and crustaceans from Oregon bays, estuaries, and inshore areas; (2) potential public health hazards from shellfish containing carcinogenic by-products of petroleum; (3) incidence of neoplastic diseases among these shellfish and possible correlation of these diseases and carcinogenic disorders; (4) metabolic pathways where benz[a] pyrene could be detoxified or modified; and (5) acute and chronic effects of selected chemical carcinogens on molluscan and crustacean gametes and ecological consequences of these effects.

Forty-four sites were sampled in 1977; molluscs were found to be contaminated by BP at 40 sites. Future research will examine possible correlation between cellular proliferative disorders and BP concentrations in molluscs.

Metabolism of Carcinogens by Marine Animals

W.P. SCHOOR, Research Aquatic Biologist

Chemical carcinogens entering the marine environment can be accumulated by seafood consumed by man, thereby providing another route of exposure. In addition, physico-chemical and biological alterations of these compounds may enhance or diminish their carcinogenic activity. The classic carcinogen, 1,2-benzopyrene, for example, is oxidized to an epoxide by microsomal oxygenase systems before it becomes the actual carcinogen.

Studies conducted at ERL,GB in 1977 attempted to determine whether or not estuarine and marine organisms can activate chemical carcinogens from pro-carcinogens to true carcinogens like their mammalian counterparts.

During this reporting period, the microsomal oxygenase systems of the mullet (Mugil cephalus) were induced by intraperitoneal injection of 30 mg/kg 3-methylcholanthrene in corn oil. After 48 h, the animals were sacrificed, and the oxygenase system was isolated by centrifugation. Incubations were carried out by using 10-40 µg of 1,2-benzopyrene or chrysene per mg of microsomal protein. The mixtures were extracted with ethyl acetate; samples were analyzed by high pressure liquid chromatography. This work demonstrated the ability of the mullet to metabolize 1,2-benzopyrene or chrysene in a manner analogous to the rat.

Future work will include different species of fish, crustaceans, and molluscs. Efforts will be made to elucidate the mechanism of induction of the microsomal oxygenases and the conjugation of metabolites. The induction studies will be complemented by studies of proliferative effects on the smooth endoplasmic reticulum of the liver.

Mechanisms of Organophosphates in Shrimp

W.P. SCHOOR, Research Aquatic Biologist

Toxicity of organophosphates in vertebrates has been linked to acetyl cholinesterase (AChE) inhibition of nerve transmission. Because the neuromuscular system of crustacea differs from that of vertebrates, shrimp response to AChE inhibitors may differ from the response of vertebrates.

In vivo tests with brown shrimp (Penaeus aztecus) showed a 96-h LC50 of 1.9 µg/ℓ for methyl parathion (MPT) and 13.6 µg/ℓ for methyl paraoxon (MPO). The only significant depression of AChE activity in the ventral nerve cords of the exposed shrimp was found in moribund shrimp at an exposure of 1.3 µg/ℓ MPT. In vitro inhibition of the isolated nerve cords was about 10% after 1 h exposure to 160 mg/ℓ MPT, and about 60% after 1 h exposure to 22 µg/ℓ MPO.

MPO was less toxic to shrimp than MPT. AChE inhibition was found only in moribund animals exposed to MPO, suggesting that toxicity in shrimp is not linked directly with AChE inhibition or that AChE activity is of little significance in shrimp.

The reason for the low toxicity of MPO in vivo could be due to its rapid degradation before reaching the active site. The specific active component for this inhibition may not be present in the nerve cord, and consequently MPO may be highly toxic only in vitro where nerve preparations are directly exposed.

The relative toxicities of MPT and MPO in vitro were the same as those observed in other systems: the MPO is 100 to 1000 times more toxic. However, neither the LC50s nor the in vivo AChE inhibition of MPT and MPO seem to agree. The reasons for high toxicity of MPT in shrimp cannot be ascertained without further study.

Determination of the Site(s) of Action of Selected Pesticides by an Enzymatic-Immunobiological Approach

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An antibody was produced to an organochlorine pesticide that inhibits ATPase enzymes, an important mechanism in the generation of energy, to gain insight into the nature of the mechanism of action of certain pesticides.

The pesticide Kelevan, the condensation product of ethyl levulinate and Kepone, was used as a hapten for covalent conjugation to various protein atigens to produce an antibody against the pesticide, thereby blocking its activity. Kelevan was successfully conjugated to bovine serum albumin (BSA), fibrinogen (BF), and gamma globulin (BGG); rabbits and chickens preimmunized with BSA and then immunized with BSA-Kelevan produced antibodies to both the hapten (Kelevan) and the carrier protein (BSA).

The antiserum to Kelevan protected ATPase activity against Kepone and its derivatives. The titer of antibody to Kelevan was critical because antiserum containing only trace amounts of Kelevan antibody failed to provide protection against its toxicity.

The antibody was concentrated by Na_2SO_4 fractional precipitation of the antiserum and obtained in pure form by affinity chromatography by using BGG-Kelevan covalently linked to Sepharose-4B. Pure antibody was obtained from untreated blood serum or plasma with no prior pretreatment or fractionation by using the BGG-Kelevan affinity column.

Complete protection of mitochondrial Mg^{2+} ATPase activity from in vitro inhibition of Kepone was obtained by a 1.2 mg quantity of Na_2SO_4 fractionated antibody and 120 µg of pure antibody; ATPase inhibition was readily reversed, as predicted, when the antibody was added prior to addition of substrate to the reaction mixture. This indicates that this procedure blocked the toxicity of Kepone to the Mg^{2+} ATPase system.

The three-year investigation will conclude February 15, 1978, after tests to determine if the antibody prevents inhibition by other chlorinated hydrocarbon pesticides. Results will be published in the next reporting period.

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